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EFFECTS OF X-RAYS ON CHROMOSOMES OF LILIUM LONGIFLORUM DURING MEIOSIS[1]

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STUDIES of the types of aberrations induced in chromosomes by irradiation during the meiotic prophase have been infrequent. The complications introduced by synapsis and crossing over make the analysis much more difficult than is the case for mitotic divisions on which most of the work has been concentrated. However, in the assessment of radiation damage to genetic material, the differential sensitivity of the meiotic stages and a knowledge of the types of aberrations expected may be of considerable importance.

SINGLETON and CASPAR (1954) have shown that the mutants recovered in *Zea mays* after irradiation vary strikingly with the stage of gametogenesis irradiated. Earlier, SPARROW (1944, 1951) had observed remarkable changes during meiosis in the sensitivity of Trillium chromosomes to the production of aberrations by irradiation. GLASS (1955) has reviewed the variations in mutants recovered after irradiating Drosophila at various stages of gametogenesis. One of the difficulties encountered in most material is the inability to predict with accuracy the stage of nuclear development at the time of irradiation. In periods where rapid shifts in sensitivity occur, determination of stage irradiated becomes an important consideration.

By employing the anthers of *Lilium longiflorum*, in which a close relation exists between the stage of gametogenesis and bud length, predictions of stage within narrow limits can be made. The large anthers provide thousands of cells at similar stages of development. Although there are twelve pairs of chromosomes, these are large and may be well-spread in squashes of relatively large pollen mother cells. Utilizing this material a study of the differential sensitivity of the chromosomes to radiation has been made for premeiotic and meiotic stages. An analysis of the types of aberrations was attempted with particular reference to the types that could be used to distinguish between chromosome, chromatid, and subchromatid breakages. The data are presented with interpretations that relate to such topics as time of chromosome duplication, crossing over and possible causes for variations in sensitivity.

MATERIALS AND METHODS

Anthers of *Lilium longiflorum* Thunb. (commercial var. "Croft") were used in these experiments. The bulbs were stored in moist peat moss at 0°C and planted in pots in the greenhouse at regular intervals.

[1] This paper is from a dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, in the Faculty of Pure Science of Columbia University.

The source of X-rays was a medical type instrument with a tungsten target operating at 10 ma at a peak potential of 75 kv. The focal distance was 20.5 inches. The radiation was unfiltered except for the inherent filtration of the unit which is equivalent to 0.5 mm of aluminum. Under the above conditions the X-ray unit delivered 40r per minute, as measured by a Victoreen dosimeter. Dosages of 15r, 30r and 60r were utilized. Exposures were made in air at a temperature of approximately 25°C.

Microsporocytes ranging in development from premeiotic interphase to metaphase I of meiosis were treated with X-rays. The effects of irradiation at the various stages of the nuclear cycle were analyzed at anaphase I. In addition, for detection of reciprocal translocations, a series of buds were analyzed at late diplotene or early diakinesis.

A close correlation between bud length and stage of microgametogenesis was demonstrated by ERICKSON (1948). In the present study the estimate of stages of development was based on the stage and bud-length correlations reported for the "Croft" variety of *Lilium longiflorum* by TAYLOR and MCMASTER (1954). The following additional factors or conditions were also considered in making the estimate of stages irradiated. (1) When groups of plants were irradiated during the same hour, the interval between irradiation and fixation aided in arranging the buds in sequence of stages. (2) Since a regular gradation in stage occurs along the length of the locule, with cells at the tip 8–16 hours in advance of those at the base, each one third of an anther was handled separately in preparing slides. Cells at the same level in either the four locules of an anther, or in the different anthers of a bud, seldom varied more than two to three hours in stage of division. (3) Seasonal variations were taken into account. For instance, in February cells reached metaphase I when the buds were 24 mm in length, while by June the same stage was found in buds 22.5–23.0 mm long.

The anthers were fixed in Carnoy's fixative (alcohol-acetic acid, 3:1), hydrolyzed in N HCl for seven minutes at 60°C and squashed in 45 percent aqueous acetic acid containing two percent haematoxylin.

Data were collected from the following groups of buds.

1. Buds (7–24 mm) were X-rayed on the plant with a dose of 30r. These plants were kept in the greenhouse until the anthers were fixed. To get a more precise estimate of the stage when cells were irradiated at late prophase, a few buds (19–24 mm) were opened by slitting along the side. One anther was removed and examined for stage of development. The opening was lightly plugged with moist cotton to minimize desiccation, and the plant was kept at 25°C until the anthers were fixed. Control material was provided from plants kept in the greenhouse as well as from nonirradiated buds opened as described above. For scoring sensitivity at anaphase I, the dose of 30r provided an optimum number of aberrations per cell.

2. Sixteen buds ranging in stage of development from premeiotic interphase to early pachytene (7.5–16.5 mm) were X-rayed on the plant with a dose of 60r. The plants were kept in the greenhouse and the anthers fixed when the cells

in the median region of the anthers reached early diplotene to late diakinesis. The cells at the upper extremity of the anthers were usually in anaphase I. Cells were analyzed both at diplotene and anaphase I. The cells analyzed at anaphase I were, when irradiated, at a stage several hours in advance of those analyzed at diplotene. However, at most stages the effects observed at anaphase I would serve as a measure of the relative sensitivity of the cells analyzed at diplotene. The only stage at which much discrepancy may occur is at preleptotene where rapid shifts in sensitivity have been observed.

The relatively high dose of 60r increases the occurrence of the less frequent types of aberrations; the analysis of cells treated with this dose were, therefore, useful for the detection of certain aberrations the presence or absence of which at particular stages of the nuclear cycle may indicate whether chromosome or chromatid breaks were induced by irradiation.

OBSERVATIONS AND RESULTS

I. Types of aberrations

In meiosis, the occurrence of crossing over between the time of irradiation and observation at anaphase I, may alter configurations to such an extent that the original aberration cannot be deduced with certainty. Thus, it is obvious that different kinds of aberrations may produce at anaphase I similar or identical configurations. It seemed necessary, therefore, to score them on the basis of appearance at the time of observation, rather than on the manner of origin.

A. Aberrations at Anaphase I

1. *Chromosome bridge with two fragments:* This may involve either homologous (Figure 1) or non-homologous (Figure 2) diads. In the former case, the original aberration may have been (a) a centric ring chromosome, (b) a paracentric inversion (inversion in one arm of a chromosome) or (c) a dicentric between homologues. A chromosome bridge between non-homologous diads may form only from a dicentric involving non-homologues.

2. *Chromatid bridge with an accompanying fragment:* The bridge may involve homologous (Figure 3) or non-homologous (Figure 4) diads. The former may result from (a) a paracentric inversion, (b) a dicentric between homologues where one of the fragments (formed by duplication of the original chromosome fragment) is involved in chiasma formation, (c) an isochromatid break with rejoining of the centric chromatids, (d) a centric chromatid ring, (e) a paracentric inversion in one chromatid of a diad, or (f) a chromatid dicentric involving homologous diads. A chromatid bridge between non-homologous centromeres may result only from a chromatid dicentric between non-homologues, where the diads concerned have moved to opposite poles.

3. *Chromatid dicentric and fragment:* Here the centromeres of two non-homologous diads, which are at the same pole of the anaphase spindle, are connected by a chromatid; the dicentric is associated with only one fragment (Figure 17). The origin of this type is the same as that of a chromatid bridge and

fragment between non-homologous chromosomes (Figure 4). In one case the diads involved have gone to opposite poles (Figure 4), while in the other they are at the same pole (Figure 17). The frequency of occurrence of each of these types would be equal.

4. *Chromatid bridge and dicentric with two fragments:* Two of the centromeres are at one pole while the third is at the opposite pole; the bridge between the two poles connects non-homologous centromeres (Figure 5). Such a configuration may originate from a chromosome dicentric involving non-homologous chromosomes.

5. *Chromatid loop with a fragment:* Two chromatids of a diad are united to form a "loop." The diad with the loop may be symmetrical or asymmetrical. A symmetrical diad with a loop (Figure 6) may be the consequence of (a) a paracentric inversion, (b) a dicentric between homologues, where one of the two fragments (formed by duplication of the original chromosome fragment) is involved in chiasma formation, (c) isochromatid break with rejoining of the centric chromatids, or (d) a chromosome dicentric involving homologous chromosomes. An asymmetrical diad (Figure 7) may result from a centric chromatid ring.

6. *A pair of chromatid loops:* Loop is at each pole with two accompanying fragments. Symmetrical diads with loops (Figure 8) may be the ultimate appearance of (a) paracentric inversion, (b) a chromosome dicentric involving homologous diads, or (c) isochromatid break and reunion in homologous arms of a pair of chromosomes.

7. *Chromatid bridge and loop with two fragments:* Here two chromatids of a diad are united to form a loop, while one of the remaining two chromatids is involved in a chromatid bridge with its homologue (Figure 9). The diad may be symmetrical, in which case the configuration may be derived from a chromosome which has a paracentric inversion in one arm and an isochromatid break with reunion of the centric chromatids in the other. The asymmetrical configuration may result from a centric ring chromosome. It is not always possible to distinguish between these two types visually.

8. *Pair of heteromorphic diads, one at each pole accompanied by a symmetrical fragment:* The pair of diads may be symmetrical (Figure 10) or asymmetrical

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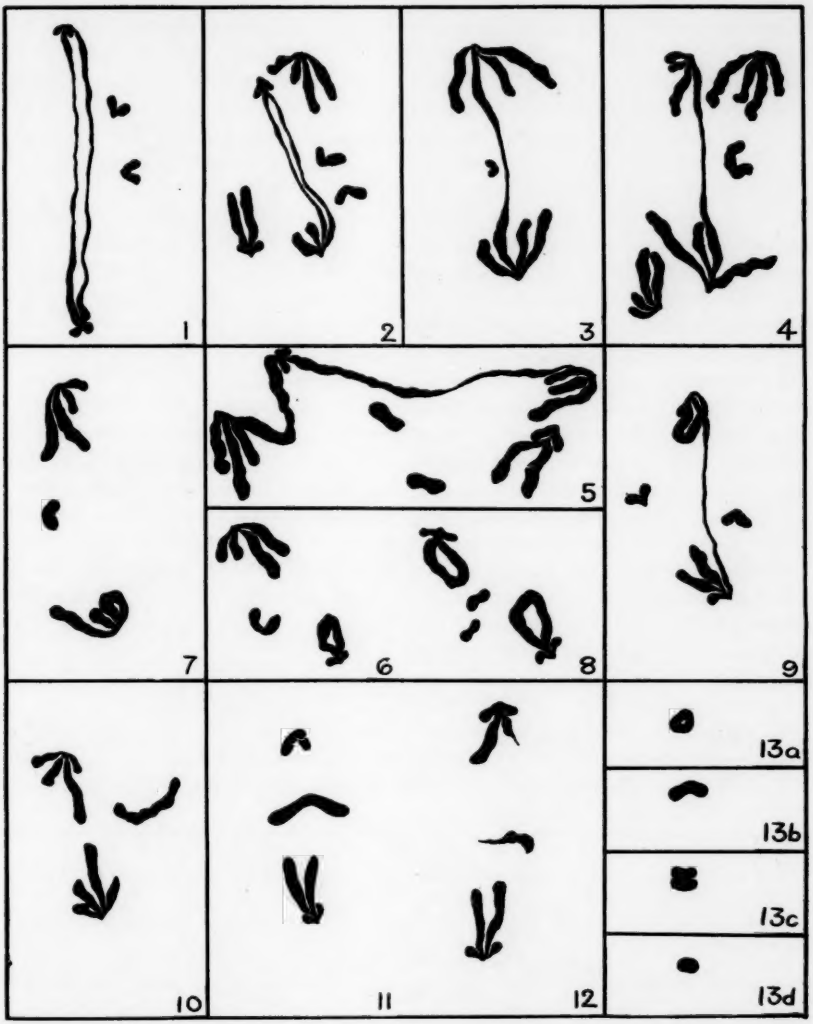
FIGURES 1–13.—Camera lucida drawings of chromosomes of *Lilium longiflorum* microsporocytes at anaphase I (×1300). FIGURE 1.—Chromosome brige between homologues, with two fragments. FIGURE 2.—Chromosome bridge between non-homologues with two fragments. FIGURE 3.—Chromatid bridge between homologues with one fragment. FIGURE 4.—Chromatid bridge between non-homologues with one fragment (DCN; see section III of Observation and Results). FIGURE 5.—Chromatid bridge and dicentric with two fragments. FIGURE 6.—Chromatid loop with one fragment. The diad with the loop is symmetrical. FIGURE 7.—Chromatid loop with one fragment. The diad with the loop is asymmetrical. FIGURE 8.—A pair of chromatid loops with two fragments. FIGURE 9.—Chromatid bridge and loop with two fragments. FIGURE 10.—A pair of symmetrical diads, one at each pole, with one fragment. FIGURE 11.—A pair of asymmetrical diads, one at each pole, with one fragment. FIGURE 12.—A pair of homologues showing a chromatid deletion. FIGURE 13.—(a) an acentric ring; (b) a rod fragment; (c) a pair of identical rod fragments; (d) a "dot" fragment.



(Figure 11). Both of these types may be obtained from (a) a chromosome terminal deletion, where the broken ends of the duplicated fragment have joined, or (b) an isochromatid break in one diad with union of the broken ends of the acentric fragments.

9. *Fragments:* These include rods, acentric rings and minute deletions (which will be referred to as "dots"), whose structure cannot be seen clearly. A rod

fragment may be (a) a chromosome, or (b) an isochromatid deletion where the broken ends of the duplicated fragments have united; it may also be (c) a chromatid deletion (Figure 12). However, the different types of rod fragments (Figures 13b, 13c) cannot always be distinguished visually. Acentric rings (Figure 13a) may result from interstitial deletions. The majority of "dots" (Figure 13d) probably are minute interstitial deletions in the form of rods or rings or very small terminal deletions (RICK 1940).

10. *Subchromatid bridges:* In this configuration the chromatids of two diads are connected interstitially with the parts of the chromatids distal to the point of connection extended nearly at right angles to the bridge; there is no accompanying fragment. The bridge may be formed between homologous (Figure 14) or non-homologous (Figure 15) centromeres. These bridges persist until late anaphase and even into telophase. However, the bridge is often ruptured mechanically during the anaphasic movement. They can be distinguished from "sticky" bridges. Nuclei showing "sticky" bridges exhibit a clumping of chromatin material or a general "stickiness" of the chromosomes in contrast to subchromatid bridges, where the bivalents are well separated showing no tendency of adhering to each other. Also, the parts of the chromatids distal to the point of attachment are closely appressed or paired in "sticky" bridges, but are separated (even at an angle of 90°) in subchromatid type aberrations. Subchromatid bridges may originate either (a) as a reciprocal translocation between subchromatids of adjacent chromatids, or (b) as a subchromatid dicentric with a subchromatid fragment (Figure 16). The close association of the subunits would prevent dissociation of the fragment at anaphase I.

B. Aberrations at late diplotene

At early diplotene the bivalents tend to aggregate after fixation, while at late diakinesis or metaphase I the chromosomes are very much condensed. Both these stages are, therefore, unsuitable for complete analysis of the structure of each

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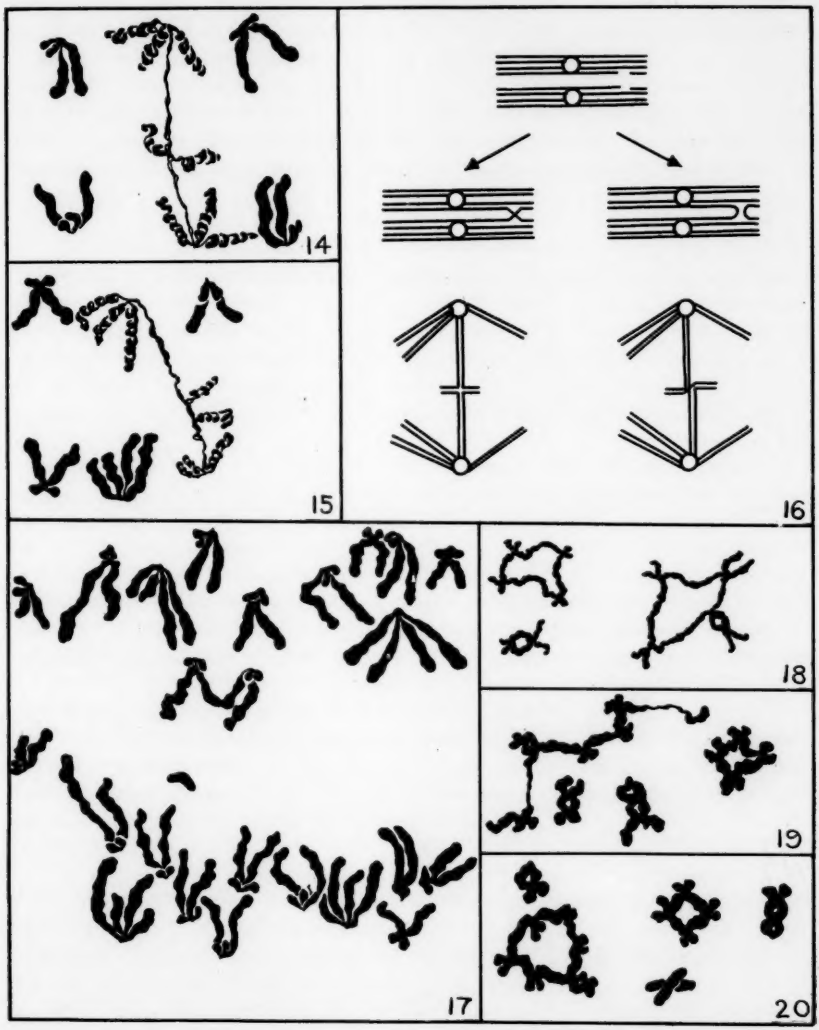
FIGURE 14.—A part of a chromosome complement of *Lilium longiflorum* at anaphase I showing a subchromatid bridge between homologous chromosomes. (×1300).

FIGURE 15.—A part of a chromosome complement of *Lilium longiflorum* at anaphase I showing a subchromatid bridge between non-homologous chromosomes. (×1300).

FIGURE 16.—Diagram indicating possible modes of origin of a subchromatid bridge: (right) a subchromatid dicentric with a subchromatid fragment; (left) a reciprocal translocation between subchromatids of adjacent chromatids.

FIGURE 17.—A full complement of chromosomes of *Lilium longiflorum* at anaphase I showing a dicentric chromatid between non-homologous chromosomes with one fragment (DCN). The diads involved are at the same pole.

FIGURES 18–20.—Camera lucida drawings of parts of chromosome complements of *Lilium longiflorum* microsporocytes showing in each case a natural translocation and radiation-induced translocations (×1300). FIGURE 18.—Late diplotene, with one induced translocation (ring of four). FIGURE 19.—Diakinesis, with two induced translocations (chain of six). FIGURE 20.—Diakinesis, with two induced translocations (ring of six).



chromosome. At late diplotene or early diakinesis the bivalents spread throughout the nucleus and are distinctly separate from each other. The chromatids are still very intimately associated, so that it is not possible to identify at this stage all the types of aberrations that show up at anaphase I. The fragments which were recognizable were of the chromosome type. A chromosome dicentric with an accompanying fragment could be detected occasionally.

The only aberration which was consistently recognizable at diplotene was the reciprocal translocation. The clone of *L. longiflorum* studied has a naturally occurring translocation which appears as a conspicuous quadrivalent (ring or chain) in every dividing cell, in both control and irradiated material. The presence of this translocation in every cell served as a guide for the recognition of the radiation-induced configurations. The translocations appeared as rings and chains of four or six chromosomes (Figures 18, 19, 20). The naturally occurring aberration was not recorded in scoring.

II. Relative sensitivity

As configurations observed at anaphase I may be the outcome of aberrations produced by one or two breaks, it was necessary to adopt a system for the comparison of relative sensitivity of the chromosomes at different phases of the nuclear cycle, which will not necessitate the classification of each aberration according to the number of breaks necessary to have produced it. Therefore, the types of aberrations observed at anaphase I have been grouped into three general categories.

Category I includes the aberrations which exhibited a chromatid connection between two or more centromeres, and those which formed centric loop chromatids. This group represents aberrations involving two breaks. On this basis, each of the types (a) chromatid bridge with fragment, (b) chromatid dicentric at one pole with fragment, and (c) centric loop chromatid with fragment, was considered as one aberration while the types (a) chromosome bridge with two fragments, (b) loop and bridge configuration with two fragments, and (c) chromatid bridge and dicentric with two fragments, were each designated as two aberrations.

The second category is a total of all fragments not involved in category 1. This includes the fragments associated with the heteromorphic diads, and those rods, acentric rings and dots whose "origin" could not be discovered. It is obvious that this is a heterogeneous group which embraces aberrations formed as a result of both one or two breaks. This category will hence be referred to as the "unclassified fragments".

The third category includes only subchromatid bridges.

Figure 21 is a graphical representation of the data obtained by the treatment with 30r (Table 1). The number of aberrations per hundred cells have been plotted against the stages of the nuclear cycle ranging from premeiotic interphase to very late diakinesis and metaphase I (three hours before anaphase I). The subchromatid bridges, which are partial chromatid breaks in contrast to the other types of aberrations mentioned above, have been represented in a separate column. For convenience, they will be considered separately.

There appear to be two extremely sensitive stages in the course of the nuclear cycle studied. The first, which is the more sensitive of the two, is at that part of premeiotic interphase which immediately precedes leptotene and which has been referred to as "preleptotene" by TAYLOR and MCMASTER (1954). The second

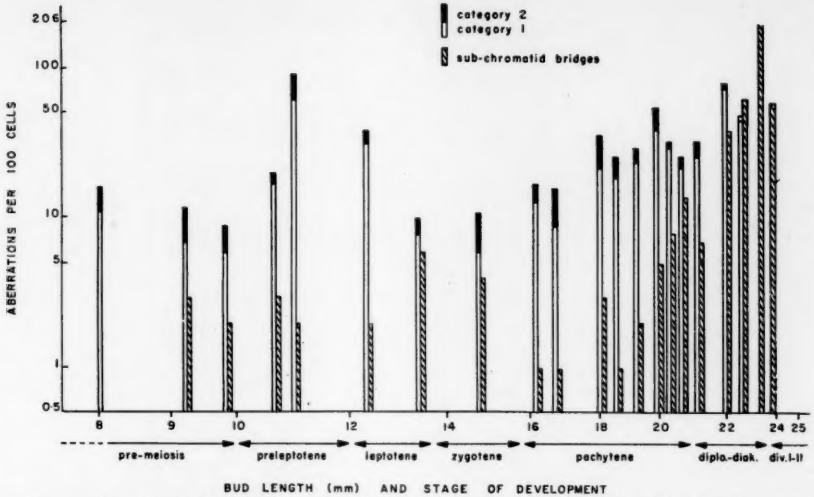


FIGURE 21.—Graph showing the sensitivity of chromosomes irradiated with 30r at various stages from premeiosis to metaphase I.

peak is at late diplotene and early diakinesis. Most of the aberrations at these two stages consist of those which were produced by at least two breaks. The proportion of "unclassified fragments" to the two-break aberrations of category 1 shows quite a range of variation at the different stages. For example, in the case of the two prominent peaks mentioned above, the ratio of unclassified fragments to category 1 aberrations is about 1:2 at preleptotene, but only about 1:16 at late diplotene. At midpachytene, however, in the 18 mm bud, the two types are almost equal.

The percentage of subchromatid bridges is not significantly different from that found in control material when cells were X-rayed up to late pachytene. The values begin an upward trend from very late pachytene or early diplotene, attain a very high point at diakinesis and then decrease toward anaphase I. In buds irradiated three and six hours before anaphase I only subchromatid type aberrations were produced.

For a more thorough analysis of the various types of aberrations a series of buds was irradiated with a dose of 15r units and floated on a culture solution (TAYLOR 1950) for a period 6–12 hours before fixing at anaphase I. Data obtained from this group of buds have been omitted here as the trends observed were similar to the ones exhibited by the experiments with 30r.

III. Change from chromosome to chromatid and subchromatid types of aberrations

Analysis of cells (irradiated with 60r) at diplotene and anaphase I provided information on the occurrence of reciprocal translocations and of chromatid

TABLE 1

Data obtained from analysis of aberrations observed at anaphase I in cells irradiated with 30r

Date of irradn.*	Irradn. to fixation (hr.)	Bud length (mm.)	Estimated stage	Types of aberrations												Total cells scored	Affected cells (%)	Categ. 1 (%)	Categ. 2 (%)	Subchrd. bridge (%)
				Fig. 1	Fig. 2	Fig. 3	Fig. 4	Fig. 17	Fig. 5	Fig. 6	Fig. 7	Figs. 8,9	Figs. 10,11	Figs. 12,13	Figs. 14,15					
2/25	338	8.1	premeiosis	..	..	9	..	..	..	2	..	..	..	5	..	100	15	11	5	0
4/14	297	9.4	premeiosis	..	..	5	..	..	1	..	..	..	..	10	3	100	17	7	5	3
4/8	290	11.8	premeiosis	1	..	4	..	..	..	..	..	..	..	3	2	100	8	6	3	2
4/14	272	10.4	preleptotene	2	..	22	..	..	..	..	..	..	1	4	5	150	18	17	3	3
4/8	278	10.7	preleptotene	..	..	51	..	1	..	11	..	..	4	28	2	100	54	63	32	2
4/14	206	12.8	early leptotene	..	..	26	4	..	..	7	..	..	2	6	2	115	29	32	7	2
4/8	166	14.5	v.late leptotene —early zygotene	..	..	5	1	1	..	1	..	..	..	2	6	100	15	8	2	6
4/8	145	14.9	zygotene	..	..	5	..	1	..	..	..	..	..	5	4	100	14	6	5	4
4/14	139	17.2	early pachytene	..	..	17	4	5	..	2	..	1	..	9	2	224	17	13	4	1
2/25	120	16.3	early pachytene	..	..	7	..	2	..	..	..	..	..	7	1	100	14	9	7	1
5/1	96	18.0	mid pachytene	..	..	30	3	5	..	..	1	..	..	24	6	174	36	22	14	3
5/14	63	18.1	mid pachytene	..	..	13	1	2	..	1	..	1	5	2	1	100	25	19	7	1
3/8	76	18.7	late mid-pachy.	..	..	15	2	3	..	3	1	..	..	6	2	100	28	24	6	2
2/12	69	20.0	early late-pachy.	..	..	40	4	1	..	..	..	..	..	17	6	113	50	40	15	5
5/12	49	21.1	late pachytene	..	..	21	8	6	1	1	..	..	1	3	11	130	32	30	3	8
3/8	45	21.8	late pachytene	..	..	17	..	2	..	1	..	1	..	4	14	100	31	22	4	14
6/15	23	22.3	mid diplotene	..	..	16	4	2	1	2	..	..	4	3	7	100	33	26	7	7
5/12	15	23.3	early diakinesis	..	..	55	13	6	..	1	..	..	2	3	39	100	70	75	5	39
6/3	13	22.8	mid-late diak.	..	..	46	..	..	..	..	..	..	..	3	64	100	73	46	3	64
6/3	6	22.8	mid-late diak.	..	..	..	..	..	..	..	..	..	..	..	103	50	88	0	0	206
5/12	3	23.7	metaphase I	..	..	..	..	..	..	..	..	..	..	..	60	100	45	0	0	60
control (average)				..	..	6	..	..	..	..	..	..	..	4	33	900	4.8	0.7	0.4	3.7

* Experiments performed in 1954.
The Figures and Numbers given as heads of columns under Types of aberrations refer to respective figures shown in the Plates of camera lucida drawings.

dicentrics between non-homologous centromeres (DCN), respectively. A reciprocal translocation represents a chromosome-type of aberration (Figures 18, 19, 20), whereas a DCN is an indication of a chromatid exchange (Figure 17).

The data obtained in this series are summarized in Table 2, and illustrated graphically in Figure 22. The disappearance of chromosome-type exchanges is gradual; but the appearance of chromatid types is rather abrupt. Reciprocal translocations are relatively numerous in cells irradiated at preleptotene, after which their number decreases until they cease to occur sometime after early leptotene, but certainly before zygotene.

There is a complete absence of DCN when irradiation occurs at early preleptotene (10.5 mm bud), as well as at the preceding premeiotic stages. These aberrations were first observed in cells irradiated at midpreleptotene (11.3 mm bud). The frequency of DCN varies with the sensitivity of the stage X-rayed, but the proportion of these rearrangements in relation to category 1 aberrations remains roughly the same from the stage in the 11.5 mm bud to early pachytene (Table 2).

The middle column in Figure 22 indicates the relative sensitivity of the 16 buds treated with 60r, based on analysis of cells at anaphase I.

Scoring of subchromatid bridges at anaphase I (Table 1; Figure 21) gives an indication of the time when four-partite chromosomes are first detected. A significant rise in the percentage of subchromatid exchanges appears at late pachytene. The value sharply increases until it reaches a peak at late diakinesis, and

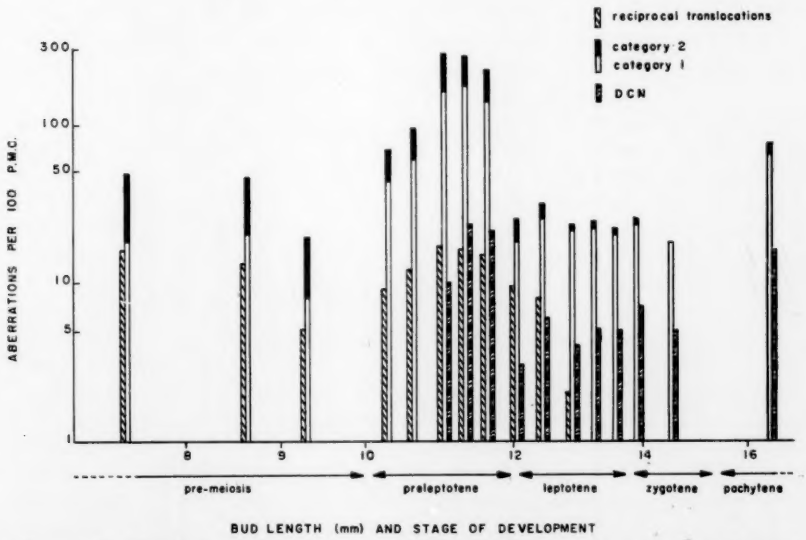


FIGURE 22.—Graph showing the stage at which the change from chromosome to chromatic type aberrations occurs, and the relative sensitivity of the cells analyzed.

TABLE 2

Data obtained from analysis of aberrations scored at diplotene and anaphase I in cells irradiated with 60r

Date of irradn.*	Irradn. to fixation (hr.)	Bud length (mm.)	Estimated stage	LATE DIPLOTENE							ANAPHASE I			
				Ring		Chain		Total cells scored	Total R.trans.	% R.trans.	% DCN	% Categ. 1	% Categ. 2	Total cells scored
				of 4	of 6	of 4	of 6							
4/16	393	7.5	premeiosis	48	0	32	0	500	80	16	0	18	30	475
5/29	362	8.8	premeiosis	45	0	21	0	500	66	13	0	20	25	400
4/16	336	9.5	premeiosis	27	0	0	0	500	27	5	0	8	11	500
5/30	295	10.3	premeiosis	36	2	7	0	500	47	9	0	43	25	500
4/16	273	10.5	preleptotene	45	2	5	3	500	60	12	0	59	34	500
5/30	207	11.3	preleptotene	23	2	55	1	500	84	17	10	160	129	500
5/30	207	11.5	preleptotene	61	0	19	0	500	80	16	23	173	93	500
5/29	204	11.7	preleptotene	56	1	14	2	500	76	15	21	138	80	500
4/16	201	12.0	leptotene	11	10	13	0	500	44	9	3	18	7	500
5/29	176	12.5	leptotene	35	0	3	0	500	38	8	6	25	6	500
5/30	169	13.1	leptotene	7	0	1	0	500	8	2	4	21	2	500
5/29	169	13.3	leptotene	0	0	2	0	500	2	0	5	22	2	450
5/29	169	13.5	leptotene	0	0	0	0	500	0	0	5	20	1	430
5/29	159	13.7	early zygotene	0	0	0	0	500	0	0	7	23	2	500
5/30	152	14.3	zygotene	0	0	0	0	500	0	0	5	17	0	500
4/16	110	16.5	early pachytene	0	0	0	0	500	0	0	16	65	11	500
	control (average)			0	0	0	0	1000	0	0	0	0.6	0.4	1000

* Experiments performed in 1954.
For explanation of Categ. 1 and Categ. 2 see text discussion of categories.

then drops somewhat toward metaphase I. The ratio of sub-chromatid to chromatid type exchanges decreases rapidly until at late diakinesis and metaphase I (three and six hours before anaphase I) the aberrations are exclusively of the subchromatid type. The experiments employing a dose of 15r also showed that two hours before anaphase I only subchromatid type aberrations were obtained.

DISCUSSION

The percentages of aberrations induced after irradiation of cells at various stages of nuclear development and observed at a later stage have been employed to indicate differential sensitivity of the chromosomes at different stages of the nuclear cycle.

SPARROW *et al.* (1952) reported that, in the case of X-ray treated Trillium anthers, scoring only at anaphase I showed pachytene chromosomes to be affected much more than chromosomes treated at diplotene and metaphase I. But when cells were analyzed at microspore metaphase the latter stages appeared to be the most sensitive ones to breakage by X-rays. In *Lilium longiflorum*, however, there are two sensitivity peaks observed, one around diplotene-diakinesis and one at preleptotene although in this study analysis was made only at anaphase I. SAUERLAND (1956) who analyzed only at anaphase I also reported a high percentage of aberrations after irradiating premeiotic interphase in *Lilium candidum*.

That the greatest number of aberrations have been observed after irradiation of cells at preleptotene and diplotene-diakinesis may have some connection with the fact that these are stages in the nuclear cycle where a reorganization of nuclear material is known to occur. Such a reorganization would cause a disturbance in the spatial relationships of the chromosomes. It has been demonstrated that factors which help to increase chromosome movement increase the chances of illegitimate fusions of broken ends of chromosomes, and hence the number of aberrations which may be recognized. (SAX 1943; CONGER 1948; WOLFF and VON BOESTEL 1954). The percentage of aberrations scored at a particular stage would not then be an indication merely of the actual number of breaks induced by X-rays. It would be, in addition, a measure of the degree of rearrangement of broken chromosomes at the stage of treatment. The large values obtained after X-raying preleptotene and diplotene may thus represent a high degree of rearrangement at these two stages, and/or a high incidence of breaks initially produced by X-radiation.

Presence of radiation-induced chromosome types of aberrations at anaphase I does not necessarily indicate whether the chromosomes were single or bipartite at the time of irradiation, since both chromatids may break at the same locus and produce chromosome-type aberrations (SAX and MATHER 1939; SAX 1941; LEA and CATCHESIDE 1942; SWANSON 1943; FANO 1943, and others). This difficulty has led to the conviction that X-raying of chromosomes is not a suitable procedure for the determination of time of chromosome duplication. The present study, however, furnishes data which indicate a time of chromosome reproduc-

tion which is in agreement with data obtained by previous workers who have employed techniques other than irradiation.

Whereas the presence of an aberration which can be produced by breakage of either a whole or half a unit cannot be used to deduce whether the chromosomes were single or double during X-raying, the occurrence of a configuration which can be formed only if half a unit is broken, is good evidence of the bipartite nature of the chromosome at the time of treatment. Such a configuration is provided by the dicentric chromatid in which non-homologous centromeres are connected. For convenience this configuration has been referred to as "dicentric chromatid, non-homologous" (DCN).

The data collected in this study show that reciprocal translocations continue to occur in early leptotene, but cease soon thereafter. As mentioned above, these aberrations may be expected to occur after chromosome duplication. DCN, on the other hand, makes a sudden appearance at about mid preleptotene. This implies that chromatids become breakable by X-rays and rejoin independently of their sister chromatids just prior to leptotene. After this period, there is a gradual shift from predominantly chromosome-type aberrations to chromatid types.

It may be pointed out that DCN could be formed by breakage of a single-strand chromosome, if reunion of broken ends were delayed until after duplication of the chromosomes. This seems improbable when it is recollected that various cytologists have found broken ends of chromosomes to heal or rejoin within a relatively short period after irradiation. In most cases this period is less than an hour (Sax 1943; Lea 1947; Steffensen and Aranson 1954). The claim that broken ends can persist for a much longer interval (Lane 1951 and 1952) has not been confirmed by other workers who have attempted to repeat the experiments on which the above contention was based (Sax and Luippold 1952; Steffensen and Aranson 1954).

Two independent means for locating the period of DNA synthesis in the nuclear cycle of *L. longiflorum* microsporocytes have indicated that DNA content of sporogenous cells become double during preleptotene. These two approaches include microphotometric determinations of amounts of DNA and detection of incorporation of P^{32} into the DNA fraction of chromosomes by the autoradiographic method (Taylor and McMaster 1954). The nuclei of cells at premeiotic interphase prior to preleptotene show an amount of DNA which has been designated as 2C in arbitrary units. The value by the beginning of leptotene is 4C in every cell. At the transition period, however, the values lie between 2C and 4C. It is interesting to note that this is also the period of chromosome duplication as shown by X-ray experiments. The chromatid breaks (DCN) first appear about the end of the period of DNA synthesis, and thereafter the chromosome type of breaks gradually disappear. There is no further incorporation of P^{32} into DNA nor an increase in amount of DNA as measured by the amount of Feulgen stain in the chromosomes throughout the period of meiosis.

Sax (1941) has observed that transition from chromosome to chromatid type aberrations in pollen mitosis of Tradescantia occurs at a period 31 to 27 hours be-

fore metaphase. In other words, doubling of microspore chromosomes occurs at preprophase towards the end of postmeiotic interphase. DNA synthesis, in the same material, has been observed to come to a completion at approximately the same period (MOSES and TAYLOR 1955). The time of chromosome duplication in the generative nucleus of Tradescantia pollen, as reported by BISHOP (1950), is also consistent with the data on time of DNA synthesis obtained by MOSES and TAYLOR (1955). Doubling of chromosomes and completion of DNA synthesis appear to take place at about the same stage of the nuclear cycle.

The period of DNA synthesis as reported by HOWARD and PELC (1952), from their studies of the incorporation of P^{32} into DNA of root tip chromosomes of *Vicia faba*, has been found to coincide with time of transition from chromosome to chromatid type breakage. Employing the same material and conditions of culture, THODAY (1954) observed that the yield of chromosome breaks, which is maximal before the synthesis period, decreases sharply during the period of synthesis; isochromatid breaks increase rapidly during the synthetic period with a peak toward the end of synthesis. After the completion of synthesis the chromatid-type aberrations become more numerous and exceed the isochromatid type between the sixth and third hour before metaphase.

The correlation between the time of chromosome doubling in irradiated microsporocytes of *L. longiflorum* and the period of DNA synthesis in the same material (TAYLOR and MCMASTER 1954) further reinforces the concept that an intimate relation exists between chromosome duplication and synthesis of a major, permanent component of the hereditary material.

Several hypotheses which have been advanced in the past concerning the mechanism of crossing over are based on the assumption that chromosomes become bipartite after pairing has been accomplished. (BELLING 1931; DARLINGTON 1935; WHITE 1942); crossovers are expected to occur in the interval between pairing of chromosomes at zygotene and their separation at anaphase I. SCHWARTZ, who has revived BELLING's hypothesis on the basis of his studies of a ring chromosome in maize (1953) and of somatic crossovers between attached X chromosomes in Drosophila (1954), suggests that chromosome duplication coincides with crossing over. He has attempted to reconcile the discrepancy between the reported time of synthesis of DNA (TAYLOR 1953; SWIFT 1953) and the estimated time of chromosome doubling as required by his hypothesis by proposing that, "chromosome duplication involves simply the joining together of the genic materials which have previously been synthesized on the chromosome". However, the present experiments reveal that, in *L. longiflorum* the genetic material is already linked together (that is, the chromosomes are duplicated) prior to the beginning of meiotic prophase. The same observation was made in microsporocytes of *L. candidum* (SAUERLAND 1956) where treatment at leptotene produced almost exclusively chromatid aberrations. These data are, therefore, not compatible with those hypotheses of crossing over which assume a duplication of the chromosomes during meiotic prophase.

Evidence indicating breakage and reunion at the subchromatid level has ac-

cumulated since the first reports by NEBEL (1936) and SWANSON (1943). The apparent temporary nature of these aberrations and their occurrence only during limited periods of the division cycle has led to uncertainties and controversies about their origin. In microgametogenesis they can apparently be induced only during late meiotic prophase and the prophase of microspore and pollen tube mitoses. Although they are produced in abundance in diplotene chromosomes, the same chromosomes after meiosis are not susceptible to this type of aberration. Some authors have considered them to be exchanges between subunits of the chromatids (SLYZINSKI 1950; CROUSE 1954; LACOUR and RUTISHAUSER 1954; SAX and KING 1955), while OSTERGREN and WAKONIG (1954) regard them as connections of the matrix. They proposed that exchanges are between whole chromatids, but that the broken parts are held together by the matrix which is supposedly developing during late prophase.

Neither of the above interpretations can explain all of the available data. The idea that they are exchanges between subunits of the chromatid does not explain their failure to show up as chromatid aberrations at the succeeding divisions (ÖSTERGREN and WAKONIG 1954). However, the hypothesis of ÖSTERGREN and WAKONIG has the following deficiencies: (1) it does not satisfactorily explain why the subchromatid effects produced by irradiation of chromosomes in first meiotic prophase and metaphase survive into the second anaphase of meiosis after passage through an interphase when the matrix would be expected to disappear (HAGUE 1953; CROUSE 1954); (2) it does not account for their production at metaphase after the presumed matrix would be fully formed.

Sensitivity to the subchromatid aberrations may be related to a change in spatial relations of pre-existing units rather than the synthesis of new material. Since there is no synthesis of DNA and very little incorporation of labeled glycine into proteins at this period (TAYLOR, unpublished), there is unlikely to be a duplication of chromosomal units similar to that which occurs at preleptotene. If the change which occurs at diplotene was due to the synthesis of a permanent constituent of the chromosome, it would be difficult to visualize an alteration that would restore it to the original condition at a later stage. However, HUSKINS and SMITH (1934) and others have suggested that doubleness or singleness of chromosomes may be a physiological and reversible state. This is clearly an area where more investigation is needed.

SUMMARY

A study has been made on the effects of X-rays on chromosomes in the anthers of *Lilium longiflorum* at various stages of meiosis.

1. Cells ranging in stage of nuclear development from premeiotic interphase to metaphase I were irradiated with doses of 15 to 60r units.

2. Materials treated with 15 and 30r were analyzed at anaphase I. Relative sensitivity of chromosomes to breakage by X-rays was based on the frequency of aberrations observed at anaphase I. Data obtained from 15 and 30r experiments exhibited similar trends. Peak yields of aberrations occur in material irradi-

ated at preleptotene and late diplotene-diakinesis, with the higher peak at preleptotene.

3. Chromosomes irradiated with 60r (premeiosis to early pachytene) were analyzed both at late diplotene and anaphase I. Reciprocal translocations—seen at late diplotene—and dicentric chromatid types of aberration between nonhomologous centromeres accompanied by one fragment (DCN)—scored at anaphase I—were used as criteria for chromosome and chromatid aberrations, respectively.

4. In cells irradiated at the premeiotic stages prior to preleptotene only chromosome type aberrations were obtained; they persist in cells treated up to early leptotene but disappear before the beginning of zygotene. Chromatid breaks, on the other hand, appear abruptly about mid-preleptotene, and persist until diakinesis (six hours before anaphase I).

5. The time of chromosome reproduction, as determined by the types of aberrations in irradiated pollen mother cells, coincides with the period of synthesis of desoxyribose nucleic acid, as reported by previous workers. This time of reproduction is not consistent with hypotheses of crossing over which require a duplication of chromosomes after pairing has occurred.

6. A sudden increase in the frequency of subchromatid type aberrations was observed after X-raying at late pachytene. The yield of subchromatid bridges rises, attaining a peak at diakinesis. Two, three and six hours before anaphase I all aberrations are of the subchromatid type.

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LOCALIZATION OF THE GENETIC FACTORS RESPONSIBLE FOR THE KINETIC ACTIVITY OF X CHROMOSOMES OF DROSOPHILA MELANOGASTER[1]

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THE genetic analysis of kinetic activity in *Drosophila melanogaster* based on the fate of first anaphase bridges derived from double crossing over within heterozygous inversions made it possible for centromeres to be characterized as weak or strong. The experimental basis for the distinction between these two types was described earlier (NOVITSKI 1952) and a more detailed description of the genetic consequences of anaphase bridge formation at both first and second meiotic divisions is presented elsewhere (NOVITSKI 1955).

Centromeres derived from a normal X chromosome are weak; whereas those found on detachments of attached–X chromosomes are strong when they carry the long arm of the Y chromosome. Some centromeres carrying the short arm of the Y chromosome are weak and some are strong. In general, the method of replacing one centromere with another involves heterochromatic exchange, with a consequent alteration of the associated heterochromatin. Of considerable genetic interest would be a determination of whether the genetic results that have been attributed to "centromere activity" depend on the nature of the centromere itself, or on the constitution of the adjacent heterochromatic regions from which the centromere is ordinarily inseparable. The experiments to be described here, designed to distinguish between these two possibilities, indicate that the latter is true.

Detachments from duplication bearing females

In the first set of experiments to be discussed, the free Y chromosome ordinarily present in an attached–X female was replaced by a small duplication that includes the euchromatic tip and proximal heterochromatin of the X. The attached-X was of special construction, carrying the fertility factors of the long arm of the Y chromosome interstitially (Figure 1). Females were irradiated and detachments carrying the long arm of the Y, which were recovered in their progeny, were analyzed genetically to determine their composition; in some instances the detachment originated by exchange with the fourth chromosome

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[2] Operated by Union Carbide Nuclear Company for the United States Atomic Energy Commission.

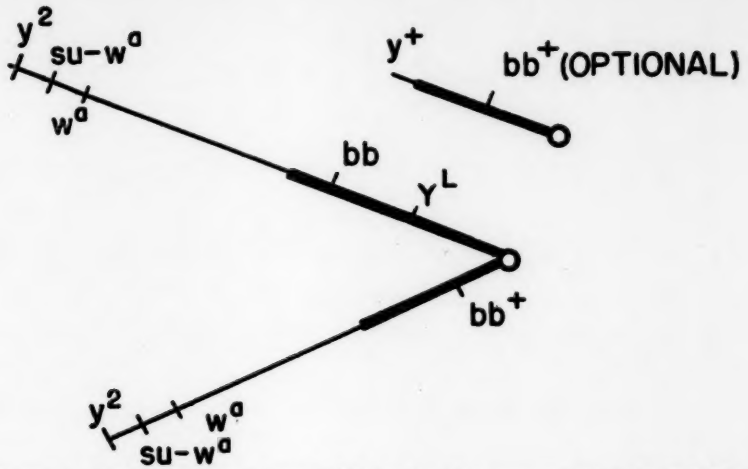

FIGURE 1.—Specially constructed attached-X carrying Y^L in one arm, and a free X duplication from which detachments used in tests were derived.

rather than with the duplication. Those detachments whose genetic composition indicated that they might prove valuable for further analysis were then tested in the usual way against both a standard weak centromere and a standard strong centromere.

When the centromeres at opposite ends of a bridge are both weak, the expected ratio of recovered male crossover progeny to patroclinous males is about 3:2; when both are strong the ratio is about 3:0; and when there is one of each type, the ratio is about 3:1. Observed ratios generally deviate from the expected owing to a slight excess of patroclinous males arising in part at least from primary nondisjunction. In principle any unknown can be characterized by a single combination with either a known weak, or a known strong. In all cases presented here, however, unknowns were tested against both a known weak and a known strong for more reliable determinations of strength. Cytological examination was made in the secondary spermatocyte, where a terminal centromere can be most readily distinguished from a subterminal one.

The method of presentation of the data in Table 1 is that used previously (NOVITSKI 1952). The structural formulas of the heterozygous females tested give the sequence of the components of the new chromosome type above the line, and the standard, with or without Y^L, below the line. The raised dot indicates the relative position of the centromere among the components of the detachment. The order of components included within parentheses is ambiguous. The cases listed will be discussed individually. Excluded from this discussion is an equal number of tests that contributed nothing essentially new to the problem.

Detachment 164–9: This detachment behaves in a typically weak fashion,

TABLE 1

Genetic results from inversion heterozygotes

Test	Maternal composition: Structural	Maternal composition: Genetic	F1, Noncrossover and double crossover regions: 0	1,2	1,3	1,4	2,3	2,4	3,4	Totals: co	Totals: Pat	Ratio as 3:x
164–9	$XY^{L}\cdot$	$y^2\ w^a$	417	0	1	2	9	7	5	43	31	3:2.1
	sc^s	$f\ v\ cv$	300	0	1	3	6	6	3			
	$XY^{L}\cdot$	$y^2\ w^a$	577	3	14	5	27	18	13	162	62	3:1.1
	$sc^s\cdot Y^{L}$	$car\ f\ v\ cv$	574	4	23	10	17	24	4			
122–19	$XY^{L}\cdot 4$	$y^2\ w^a$	1940	4	17	4	25	39	21	175	115	3:2.0
	sc^s	$f\ v\ cv$	1184	1	17	7	16	18	6			
	$XY^{L}\cdot 4$	$y^2\ w^a$	728	1	8	6	21	17	3	132	43	3:1.0
	$sc^s\cdot Y^{L}$	$car\ f\ v\ cv$	713	10	15	8	20	17	6			
179–8	$X(\cdot Y^{L})4$	$y^2\ w^a$	859	2	5	1	22	20	9	99	40	3:1.2
	sc^s	$f\ v\ cv$	489	0	11	5	10	15	3			
	$X(\cdot Y^{L})4$	$y^2\ w^a$	1035	6	13	10	27	18	10	168	11	3:0.2
	$sc^s\cdot Y^{L}$	$car\ f\ v\ cv$	650	9	28	5	27	12	4			
3–18	$X(Y^{L}\cdot)$	$y^2\ w^a$	2402	5	19	16	58	60	26	298	101	3:1.0
	sc^s	$f\ v\ cv$	1510	5	20	20	24	36	9			
	$X(Y^{L}\cdot)$	$y^2\ w^a$	1317	8	26	7	45	23	10	240	14	3:0.2
	$sc^s\cdot Y^{L}$	$car\ f\ v\ cv$	1212	12	35	7	33	19	4			
118–12b	$X(Y^{L}\cdot)$	$y^2\ w^a$	2438	5	15	10	39	27	13	202	71	3:1.1
	sc^s	$f\ v\ cv$	1542	8	22	11	28	18	6			
	$X(Y^{L}\cdot)$	$y^2\ w^a$	1037	1	11	15	22	6	1	92	9	3:0.3
	$sc^s\cdot Y^{L}$	$car\ f\ v\ cv$	891	6	14	13	18	4	0			
174–13	$XY^{L}\cdot$	$y^2\ w^a$	1015	0	6	5	19	27	15	128	69	3:1.6
	sc^s	$f\ v\ cv$	716	3	5	8	14	20	6			
	$XY^{L}\cdot$	$y^2\ w^a$	507	2	16	9	19	26	5	154	37	3:0.7
	$sc^s\cdot Y^{L}$	$car\ f\ v\ cv$	404	3	22	15	14	18	5			
9–10r	X 4-	$B\ y$	912	6	32	21	25	29	3	239	52	3:1.3
	N*	$sc\ cv\ v\ f$	1024	10	26	22	37	24	4			

	X 4-	*B y*	141	0	20	8	21	24	5	139	10	3:0.4
	N·Y^{L}	*sc cv v f*	125	3	14	8	11	17	8			
9–10e	X 4-	*B y*	3322	24	94	42	106	50	22	638	182	3:1.7
	N	*sc cv v f*	2504	22	90	43	72	55	18			
	X 4-	*B y*	967	4	36	14	26	16	5	199	23	3:0.7
	N·Y^{L}	*sc cv v f*	923	7	28	12	28	13	10			
9–10o	X 4-	*B y*	1190	7	24	6	29	23	5	189	51	3:1.6
	N	*sc cv v f*	1054	6	34	13	19	22	1			
	X 4-	*B y*	1158	6	24	8	18	18	2	165	15	3:0.5
	N·Y^{L}	*sc cv v f*	989	2	29	13	27	14	4			
9–24d	X 4-	*B y*	816	5	25	5	20	17	8	171	47	3:1.8
	N	*sc cv v f*	840	6	32	9	27	12	5			
	X 4-	*B y*	929	1	21	15	30	16	4	164	33	3:1.3
	N·Y^{L}	*sc cv v f*	817	4	15	5	28	22	3			
9–20i	X 4-	*B y*	3016	19	63	50	96	97	40	710	192	3:1.6
	N	*sc cv v f car*	2761	15	110	54	85	55	26			
	X 4-	*B y*	2062	5	47	20	62	51	26	375	103	3:1.6
	N	*sc cv v f*	1852	11	42	14	50	36	11			
	X 4-	*B y*	1517	15	73	15	71	61	24	483	70	3:1.1
	N·Y^{L}	*sc cv v f*	1379	7	60	30	65	45	17			

* N = normal sequence.

Region 1, *y-cv*; region 2, *cv-v*; region 3, *v-f*; region 4, *f-sfa*; progeny from each cross are tabulated in two rows, with the individuals in each row carrying the chromosome of the structure designated by the corresponding row in the maternal structural composition. The paternal genotype in tests 164–9 through 174–13 was *In(1)*dl-49, *y w B*; consequently, only male progeny were scored. The paternal genotype in tests 9–11b through 9–20i was *y w^{m} cv v f* and all progeny were scored. To compensate for twice as many crossovers being scored in the latter tests, the patroclinous male class has been doubled in obtaining the 3:x ratio.

giving a 3:2 (3:2.1) ratio with *sc*^{8} and a 3:1 (3:1.1) ratio with *sc*^{8}.Y^{L}. Cytologically, it has a terminal centromere, presumably an X centromere derived from the duplication, and is of interest because it represents the first case in which a detachment carrying the fertility factors of Y^{L} is not also strong.

Detachment 122–19: This case is typically weak in both tests. It arose by an exchange between the attached-X and the fourth chromosome, which resulted in a detachment that retains the centromere of the attached-X, but in which the tip of the fourth replaces the arm not carrying Y^{L}. It confirms the point made in the preceding case that the presence of Y^{L} does not necessarily result in a strong centromere and further shows that there is no inherent "strongness" to the centromere of this particular attached-X.

Detachment 179–8: Like 122–19, the tip of the fourth chromosome has replaced one of the arms of the attached-X, so that the centromere of the attached-X is retained in the detachment. Crossover tests show it, unlike 122–19, to be strong. Genetically, another difference between these two is that 179–8 carries the normal allele of bobbed whereas the preceding one carries the recessive allele. The possible significance of this will be discussed.

Detachments 3–18 and 118–12b: Both of these are clearly strong. They carry Y^{L} and cytological examination shows both to have a subterminal centromere, indicating that they still retain the centromere of the attached-X. Both carry the normal allele of bobbed.

Detachment 174–13: This is an interesting case because the ratios from both tests are abnormal, the values being intermediate between weak and strong. Ordinarily, observed ratios belonging to the 3:2 category vary from 3:2.0 to 3:2.4, those belonging to the 3:1 class vary from 3:0.9 to 3:1.4 and the 3:0 expected usually appears as a ratio between 3:0.1 and 3:0.4. As pointed out previously (NOVITSKI 1952), the distribution of these values is sharply discontinuous. The two values from 174–13, 3:1.6 and 3:0.7, are both in an atypical region of the distribution. At a later date this test was repeated on a somewhat larger scale; the results were in good agreement with those given in Table 1. The over-all ratios for the combined data were 3:1.68 and 3:0.66.

Cytological examination shows this detachment to have a terminal centromere. It seems probable that this centromere originated from chromosome four, since the duplication used to detach the attached-X chromosomes was shown later to have a centromere derived from chromosome four. It might be noted, incidentally, that intermediate values such as these may be more widespread than the present data indicate; PROFESSOR STANLEY ZIMMERING (unpublished) pointed out the occurrence of such values in experiments involving other types of chromosomes.

Two other detachments (118–2b and 122–13) were analyzed in this series; the data are not presented here because they give typically weak values and add nothing to the arguments already presented. It should be noted, however, that on genetic analysis these detachments were shown to carry the recessive allele of bobbed from the attached-X, like all the other weak ones and were unlike the strong ones, which all carried the normal allele of bobbed.

Detachments from nonduplication-bearing females

The next set of experiments to be described involves detachments obtained in a somewhat different way; an attached-X whose arms are in inverted sequence and also carry the short arm of the Y chromosome distally was used. Females with such a chromosome, but lacking a Y chromosome were irradiated, and detachments were collected. It is known that under such conditions the fourth chromosome is predominantly involved (PARKER 1954; MULLER and HERSKOWITZ 1954). The desired product was the one that would carry the centromere of the fourth chromosome, and not its tip. Consequently, matings of the detachments were made to *sv*^{n}, which genetically marks the tip of the fourth, and those detachments (numbering 25) which proved to carry its normal allele were discarded. Of 35 others, 24 showed a high production of Minute (haplo-IV) progeny, indicating that part of the fourth chromosome was involved in the detachment and was interfering with the normal disjunction of the free fourth chromosomes. Seven of these were selected for further testing, as well as one that did not produce typical haplo-IV progeny but rather did produce a weak Minute type.

Detachment 9–10r: Cytologically, this detachment has a terminal centromere. That this is the centromere of the fourth is shown not only by the production of haplo-IV progeny, but also by the fact that such haplo-IV individuals carry the detachment, indicating that one of the fourth chromosomes occasionally pairs with the detachment and disjoins from it. This insures that the class getting the homolog of the detachment will also get the fourth, whereas that class getting the detachment may or may not get the other unpaired fourth. It is clearly strong (Table 1).

Detachment 9–10e: This is the case that produces weak Minutes instead of typical haplo-IV types. It is also the one case in which we could demonstrate the normal allele of a fourth chromosome marker other than shaven; this shows that the detachment consists of the centromere and the basal section of the fourth chromosome, up to some point between the loci of *ci* and *ey*, attached to the X chromosome. The centromere is weak, although the ratios seem somewhat depressed as in 174–13.

The remainder of the tests carried out were essentially duplicates of those already described. Cases 9–10g, 9–11b, and 9–11a were kinetically similar to 9–10r, which is typically strong, but differed from it in having subterminal centromeres. Cases 9–10o, 9–20i, and 9–24d, similar to 9–10e and 174–13, appeared weak, and also gave a somewhat depressed 3:2 ratio; they have terminal centromeres.

DISCUSSION

It is now possible to present a simple, consistent picture of the contribution of the chromosome to its kinetic activity. In the first place, it is clear that centromeres as such have no specific properties as measured by the anaphase bridge tests described in this and previous works. If they did have such properties, consistent results would be expected from a given type of centromere, but in both sets of experiments described here, detachments with centromeres of similar

origin behave quite differently. In set one, both 122–19 and 179–8 must carry the centromere present on the attached-X, yet one is weak and the other strong; similarly in the second set, 9–11b and 9–10e carry the fourth centromere and behave differently.

If the centromeres of the different chromosomes of the complement had different activity, the level of this activity might be related to the physical size of the chromosome. Previous observations were consistent with this thought, since normal X centromeres were invariably weak relative to those found on detachments; whereas those that proved to be strong were derived from attached-X chromosomes and were probably ultimately derived from the Y chromosome. On this basis, the fourth might be predicted to be weaker than the X, since the small dot chromosome should not require so high a level of kinetic activity as the much larger X chromosome. It is shown, however, that the detachments with the fourth centromere may be about equivalent in strength to the X centromere (174–13, 9–10e, 9–10o, 9–20i, and 9–24d) or equivalent to the standard strong (9–10r).

It seems definite, therefore, that the differences in kinetic activity being measured in these anaphase bridge experiments cannot be referred to some property of the centromere as a point, but that its activity is being modified by its association with genetic factors. It has been shown (NOVITSKI 1952) that the kinetic property of the chromosome must reside in the vicinity of the centromere. Furthermore, when detachments are produced, this region contains indeterminate and variable amounts of heterochromatin from both the attached-X and the other chromosome participating in the detachment process. A completely reasonable conclusion is that the detachments analyzed here differ in their behavior because they contain different combinations of those elements responsible for the kinetic activity of the chromosome. The absence of suitable techniques for analyzing the heterochromatic regions of chromosomes in Drosophila makes it difficult to become more specific. Nevertheless, some of the cases do indicate that the detachment process involves a redistribution of such factors that gives rise to new situations on the detached chromosome. In the first set of experiments described here, all the detachments were tested for the presence or absence of the normal allele of bobbed. All three detachments with strong centromeres carried the normal allele of bobbed and were therefore probably duplicated for the bobbed region (see Figure 1) and conversely, the five that were not strong carried only the recessive allele. This can be considered evidence that, in this particular attached-X, a factor (or factors) imparting strength to the centromere was located so close to the locus of the normal allele of bobbed that the detachment process failed to separate them. It is conceivable that, in this test, strength is correlated with the dosage of some factor close to the locus of bobbed; however, no such correlation between the bobbed constitution and kinetic activity could be demonstrated in the second set of detachments. An explanation of this difference between the two sets of detachments may reside in the different heterochromatic constitution of the initial attached-X's.

The occurrence in these experiments of several "atypical cases" in which the ratio after test with a weak centromere is depressed somewhat below the 3:2 expectation may have some significance in this connection. 174–13, 9–10e, 9–10o, 9–20i, and 9–24d can be explained as instances in which the redistribution of the factors responsible for the kinetic activity was such that these cases represent essentially new combinations that have no counterpart in previous tests. They resemble the weak type yet consistently deviate from the 3:2 and 3:1 ratios in an unexpected direction; it should be noted that in these five cases the centromere of chromosome four has been attached to the X. It could be argued that the kinetic behavior of these centromeres is not strictly weak but is intermediate between weak and strong; thus in a certain proportion of the bridges the centromeres might behave as if they were strong. This would have the effect of shifting a 3:2 ratio in the direction of a 3:1 and a 3:1 toward a 3:0, and would fit the observations. However, the possibility is not excluded that this behavior is the result of some condition other than the one given; e.g., negative chromatid interference or an abnormally high incidence of primary nondisjunction in the females.

The data available suggest that the time of action of the system responsible for kinetic activity at the first meiotic division is subsequent to the time of genetic exchange, i.e., that the constitution of the bridge and not the constitution of the oocyte determines observed results. In an extensive series of experiments, we attempted to alter the kinetic activity of previously studied combinations by manipulating the heterochromatic constitution of the oocytes in which the bridges were formed. We modified the acentrics produced as a concomitant of bridge formation by attaching the short arm of the Y chromosome to the distal end of one or both chromosomes, or by using the terminal regions of different scute inversions. The addition of free heterochromatic duplications of X or Y origin to the heterozygous females being tested was another method used for alteration of the heterochromatic constitution of the oocyte. In no instance did the bridges behave in the way they would have if these same segments had been attached to the centromere region. The interpretation of this result is that the factors that are effective in a determination of the kinetic level are those actually associated physically with the centromere, and that those present on independent chromosomes, or on the acentric fragments produced after the exchanges that produce the bridges, are ineffective. This conclusion is similar to the one reached by RHOADES (1952) in his analysis of neocentromeres in maize. Another point of similarity in these two analyses comes from the apparent synergistic action of certain types of weak centromeres that together produce a strong effect or produce a strong effect in combination with a known strong but not with a known weak centromere (see NOVITSKI 1952). One interpretation of this effect is that there is a flow along the dicentric chromosome of some product of the centric regions from one end of the bridge to the other that enhances the activity of the weak centromeres. This resembles the movement of fiber-forming substance from the centric regions to the knobs of the chromosomes postulated by RHOADES to explain the formation of neocentromeres in maize.

SUMMARY

The genetic analysis of new X chromosome types of *Drosophila melanogaster* leads to the following conception of the genetic contribution to kinetic activity. Chromosomes do not play a passive role in their progression to the poles; they contribute autonomously to the formation of the spindle or to the process of progression, and the magnitude of this contribution differs for different chromosomes. The function of the centromere is constant from chromosome to chromosome, and it is even possible that the centromere is an inactive anchor point on the chromosome to which the spindle fiber becomes attached. The measurable differences in kinetic activity are attributable to the constitution of the heterochromatic regions of the chromosomes immediately adjacent to the centromere.

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GENETIC RECOMBINATION IN NEUROSPORA

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STUDIES of the genetic behavior of nutritional mutants of Neurospora have, in the past, been carried out with certain assumptions already in mind. These assumptions were based, in part, on conclusions drawn from the behavior of mutants of higher organisms, particularly Drosophila and maize. It has been assumed that nuclei of the vegetative mycelia are regularly haploid; that one to one segregation of mutant and wild counterparts in a small number of asci demonstrates, more probably, control of the mutant expression by a single locus; that unlinked characters recombine by reassortment of chromosomes, but that linked characters recombine through crossing over. In the course of the genetic experiments, situations encountered which appeared not interpretable in a straightforward way within the framework of these assumptions have been put aside with the hope that, as knowledge of the organism and the mutants increased, such observations would become understandable in terms of the basic assumptions. This does not appear to be taking place, however. Rather, it seems, from the observations now accumulated, that the assumptions are, in general, simply not applicable. Further, it seems that it may be possible to arrive at others which more nearly apply. Observations accumulated over a number of years will be discussed from this point of view.

Frequencies of recombinants among random spores

Counts given in Table 1, 2, 3 and 4 are of samples of spontaneously released ascospores, spread on minimal agar plates and classified and counted under the microscope about 12 hours after germination. (The spores of mature asci are shed with some force so that in a slant culture they are deposited on the wall of the tube opposite the perithecia.) Thus it can be ascertained that each individual arises from a single ascospore and the question of heterocaryosis does not arise unless the spores themselves are heterocaryotic (MITCHELL, PITTENGER and MITCHELL 1952). It may be pointed out that data obtained in this way from random spores of Neurospora have, perhaps, few parallels. The frequencies observed are, presumably, analogous to gamete frequencies.

As such data have been accumulated odd coincidences have increased in number to a point at which they are scarcely credible as coincidences. Some examples will be elaborated as illustrations. The mutant designations used are those assigned by BARRATT *et al.* (1954). In general, different numbers following the same symbol (*arg-3* and *arg-6*, for example) indicate the same or similar requirements thought to be due to mutations at different "loci."

Taking the map distance or recombination frequency between two markers as twice the percentage of germinated spores which show neither of the two mutant phenotypes has been the usual practice in mapping from random spore counts. Thus cross 51 gives the distance between *arg-3* and *lys-4* in linkage group I as 2 x 1.9 units. But cross 52 shows *arg-6* and *lys* (28815), previously assigned to positions in another part of group I, (BARRATT *et al.* 1954) also to be located 2×1.9 units apart. If these four mutants actually represent four different sites on the chromosome it is curious that *arg* should be located at the same distance from *lys* in each case. No *lys* mutant is known to be located near *arg-2* in group IV. However, it has recently been found that *pyr-3a* and *pyr-3b* will respond to lysine as a substitute for pyrimidine if ammonia is excluded from the basal medium. And curiously, crosses 64 and 65 show *pyr-3a* and *pyr-3b* to be located 2×0.62 and 2×2 units, respectively, from *arg-2*. Such coincidences in conjunction with the observations that *lys* mutants are inhibited by arginine (DOERMANN 1945), *arg* mutants are inhibited by lysine and both *arg* and *lys* mutants interact in unpredicted ways with *pyr-3a* and its suppressor (HOULAHAN and MITCHELL 1948; MITCHELL and MITCHELL 1952) suggest that the relationship between *arg* and *lys* mutants is not readily understandable in terms of entirely separate mutational effects.

Further examples of these coincidences are offered by crosses involving *ad* and *hist* mutants. Cross 77 gives the distance between *hist-4* and *ad-6* in group IV as 2×2.3 units which is rather near that obtained between several pairs of mutants in group I as follows: *hist-3* × *ad-3*, 2×2.2 (91); *hist-2* × *hist* (1710), 2×1.9 (79) and *hist-2* × *hist-3*, 2×2.2(84). Nor are these distances very different from those found from *hist-2* × *ad-3*, 2×3.3 and 2×3.4 (42 and 87) or from *hist* (1710) × *ad-3*, 2×1.2 (43). Cross 78, on the other hand, gave a different distance between *hist-4* and *ad-6*, 2×8.4, but this is almost the same as that between *ad-7* and *hist-1* in group V, 2×8.9 (44). Also, it is curious that cross 83, of *hist-3* × *sn hist-3*, not expected to give prototrophs at all, gave 7.9 percent, and one of the crosses of *hist-3* × *sn hist-3 ad-3* (94), instead of giving no prototrophs as expected, gave 17.8 percent which is very nearly the same as the frequency of prototrophs from *ad-7* in group V × *ad-6* in group IV, 17.3 percent (50) although the latter cross was expected to give 25 percent.

In general, with respect to mutants assumed to be linked, there seem to be certain preferred recombination frequencies. Of particular interest in this connection are crosses 56, 57 and 58 in which *rib-1* gave 8.2, 7.4 and 7.9 percent prototrophs with *cyt* (C117), *tryp-2* and *tryp* (B1312) respectively. But cross 61, of *rib-1* × *pyr-3d*, which have been supposed to show false linkage, gave 8.3 percent. It seems a strange coincidence that the false and true linkages should be so nearly the same. The false linkage was once thought to be associated with a reciprocal translocation carried by the original isolate of *pyr-3d*, but it was later found that this strain is characterized instead by an extra chromosome (MCCLINTOCK 1954; SINGLETON 1948).

It may be seen from Tables 2 and 4 that crosses involving the three "colonials,"

TABLE 1

Mutants crossed to wild

Cross	Prototrophs percent	Number of spores
1. Wild Abbott 4 A × *hist-2* I	42.4	823
2. Wild Abbott 4 A × *hist-3* I	33.0	2513
3. Wild Abbott 4 A × *hist-4* IV	38.6	956
4. Wild—5912–2 A × *tryp-1* III	43.5	1443
5. Wild—5912–2 A × *pdx* (37803) IV	46.6	1413
6. Wild—5912–2 A × *ad-7* V	49.3	1629
7. Wild—4323–1 a × *pdx* (37803) IV	49.4	1601

TABLE 2

Crosses of nutritional and visible mutants

Cross	Prototrophs percent‡ v+	v	Number of spores
8. *col-4* IV × *tryp-1* III	41.9	16.4	1661
9. *col-4* IV × *pyr-3a* IV*	1.2	46.9	1717
10. *col-4* IV × *pyr-3b* IV†	0.73	49.5	4113
11. *col-4* IV × *pyr 3d* IV	4.5	50.8	2879
12. *col-4* IV × *arg-2* IV*	0.4	48.9	3253
13. *col-4* IV × *pdx* (37803) IV	2.3	44.2	915
14. *col-4* IV × *arg-3* I	28.8	14.5	743
15. *col-4* IV × *ad-1* VI	23.8	24.2	959
16. *cot* IV × *ad-5* I	19.0	26.8	868
17. *cot* IV × *hist-1* V	22.2	27.0	803
18. *cot* IV × *hist-3* I	21.4	28.5	586
19. *cot* IV × *hist-4* IV	5.1	46.4	994
20. *cot* IV × *pan* (34556) IV	1.8	50.1	1508
21. *cot* IV × *ad-6* IV	0.76	55.2	787
22. *cot* IV × *ad-6* IV	0.87	58.5	1379
23. *cot* IV × *pyr-2* IV	11.2	not counted	2621
24. *cot* IV × *pyr-2* IV	11.3	not counted	3068
25. *cot* IV × *pyr-3b* IV†	12.4	not counted	3027
26. *cot* IV × *pyr* (37709) IV	13.0	not counted	1913
27. *cot* IV × *ad-7* V (25° C)	22.9	19.3	647
28. *sn* (C136) I × *arg-3* I	1.6	49.6	4902
29. *sn* I × *arg-3* I	4.0	51.1	2305
30. *hist-2* I × *sn* I	0.77	49.7	5033
31. *sn* I × *hist* (1710) I	2.1	49.1	2129
32. *sn* I × *lys-4* I	1.8	50.1	1622
33. *sn* I × *sn* I	1.9	96.5	4434
34. *sn* I × *sn-b* (C132) I	0	100	4000 app.

* MITCHELL, PITTENGER and MITCHELL 1952.
† MITCHELL and MITCHELL 1954.
‡ The symbol, v, represents the appropriate visible mutant, *col*, *cot* or *sn*.

TABLE 3

Crosses between nutritional mutants

Cross	Prototrophs percent	Number of spores
35. *tryp-1* III × *nic-1* I	27.1	1102
36. *tryp 1* III × *pdx* (37803) IV	22.4	2450
37. *pdx* (37803) IV *tryp-1* III × *tryp-1*	0	4000 app.
38. *ad-6* IV × *tryp-1* III	45.0	650
39. *tryp-1* III × *hist-1* V*	28.7	1583
40. *hist-2* I × *tryp-1* III*	27.6	553
41. *hist-4* IV × *tryp-1* III	23.9	4891
42. *hist-2* I × *ad-3* I*	3.3	1712
43. *hist* (1710) I × *ad-3* I	1.2	2694
44. *ad-7* V × *hist-1* V	8.9	906
45. *hist-4* IV × *pyr-2* IV*	13.1	2448
46. *hist-4* IV × *pyr-3a* IV*	13.9	4891
47. *pyr-3b* IV × *hist-4* IV‡	14.3	3116
48. *hist-4* IV × *hist* (1710) I	23.3	2335
49. *hist* (1710) I × *hist-3* I	0.07	5926
50. *ad-7* V × *ad-6* IV	17.3	797
51. *arg-3* I × *lys-4* I	1.9	1950
52. *lys* (28815) I × *arg-6* I	1.9	1910
53. *inost* (83201) V × *pyr-3a* IV	34.4	3776
54. *rib-1* VI × *ad-1* VI	0.45	6783
55. *rib-1* VI × *un* (66204) VI	4.2	7417
56. *rib-1* VI-*cyt* (C117) VI†	8.2	2405
57. *rib-1* VI × *tryp-2* VI	7.4	1654
58. *rib-1* VI × *tryp* (B1312) VI	7.9	869
59. *rib-1* VI × *pyr-4*	24.9	1640
60. *rib-1* VI × *hist-1* V*	31.2	1228
61. *rib-1* VI × *pyr-3d* IV	8.3	3381
62. *rib-1* VI × *pyr-3d* IV	15.4	1155
63. *un* (66204) VI × *cyt* (C117) VI	3.8	743

* HAAS *et al.* 1951.
† MITCHELL, MITCHELL and TISSIERES 1953.
‡ MITCHELL and MITCHELL 1954.

col-4, *cot* (C102, colonial, temperature-sensitive) and *sn* (C136, snowflake), with nutritional mutants linked to them show, in general, rather similar map distances. The three mutants are similar in phenotype, the abnormality consisting of excessive branching of the hyphae. At about 32°C the growth habit of *cot* is very like that of *sn* at all temperatures, whereas *col-4* differs from them both in that its hyphal branches are not straight, but gently curved. In several crosses (between nutritional mutants) in which *sn* was heterozygous and which gave low frequencies of *sn* prototrophs (particularly crosses involving *hist* and *ad*) it was noticed that some of these prototrophs were so like *col-4* that, had *col-4* been present in the cross they would have unhesitatingly been so classified. It was not a complete surprise, then, when a cross of *sn* × *lys arg sn* (not shown in the table) gave the following frequencies of prototrophs: *sn col-4*$^+$, and *sn col-4*, 52

TABLE 4

Crosses involving three mutants

Cross	Prototrophs percent‡ v+	v	Number of spores
64. *col-4 arg-2* × *pyr-3a* IV	0.60	0.018	5608
65. *col-4 arg-2* × *pyr-3b* IV	2.0	0	4542
66. *arg-2* × *col-4 pyr-3d* IV*	0.11	0.94	2653
67. *col-4 arg-2* × *pyr* (49001) IV	1.1	0	7674
68. *col-4 pyr-1* × *pyr-3b* IV	1.2	1.8	2898
69. *col-4 pyr-3b* × *pyr-1* IV†	0.75	0.23	2611
70. *col-4 pyr-3b* × *pyr-1* IV	1.7	1.4	4309
71. *col-4 pyr-3d* × *pyr* (67011) IV	0.9	0	3789
72. *col-4 pyr-3b* IV × *sn* I	23.7	0.35 *col sn*+ 25.6 *col*+ *sn* 0.31 *col sn*	2287
73. *col-4 pyr-1* × *pdxp* (39106) IV	0.036	0.14	2729
74. *cot* × *col-4 pyr-2* IV	1.42	35.9 *col*+ *cot* 7.2 *col cot*+ 7.2 *col cot*	1829
75. *pan* (34556) × *cot ad-6* IV†	0.89	0.29	3027
76. *cot hist-4* × *pan* (34556) IV†	0.67	0.04	9773
77. *cot hist-4* × *ad-6* IV†	1.1	1.2	4290
78. *hist-4* × *cot ad-6* IV	3.9	4.5	1690
79. *sn hist-2* × *hist* (1710) I	1.8	0.06	10915
80. *hist-2* × *sn hist-2 hist* (1710) I	0	0	2000 app.
81. *hist* (1710) × *sn hist-2 hist* (1710) I	0	0	3000 app.
82. *sn hist-3* × *hist* (1710) I	0.006	0.003	59000 app.
83. *hist-3* × *sn hist-3* I	5.9	2.0	1818
84. *sn hist-2* × *hist-3* I	2.0	0.17	8978
85. *hist-2* × *sn hist-2 hist-3* I	0	0	3000 app.
86. *hist-3* × *sn hist-2 hist-3* I	0	0	5000 app.
87. *sn hist-2* × *ad-3* I	3.3	0.12	4927
88. *hist-2* × *sn hist-2 ad-3* I	0	0	6000 app.
89. *hist* (1710) × *sn hist-2 ad-3* I	0	0	1000 app.
90. *hist-3* × *sn hist-2 ad-3* I	12.0	0.6	1463
91. *sn hist-3* × *ad-3* I	2.1	0.05	9558
92. *sn ad-3* × *hist-3* I	0.21	4.2	2418
93. *hist-3* × *sn hist-3 ad-3* I	19.0	2.7	1608
94. *hist-3* × *sn hist-3 ad-3* I	16.0	1.8	1279
95. *sn hist-2* × *arg-3* I	1.8	0.3	8414
96. *arg-3* × *sn hist-2* I	2.3	0.5	7915
97. *sn hist-2* × *arg-3 hist-2* I	0	0	4000 app.
98. *sn* × *arg-3 hist-2* I	0.09	53.9	3544
99. wild × *sn arg-3 hist-2* I	46.3	0.018	5674
100. *sn hist-2* × *lys-4* I	0.6	0.18	11175
101. *sn hist-2* × *lys-4* I	4.0	0.18	5400 app.
102. *sn arg-3* × *lys-4* I	2.6	5.4	6679
103. *sn* × *arg-3 lys-4* I	0.12	50.3	2449
104. wild × *sn arg-3 lys-4* I	36.7	0.11	1756
105. wild × *sn arg-3 lys-4* I	44.0	0.039	2800
106. *ad-5* × *sn arg-3* I	0.13	0	6041

* MITCHELL, PITTENGER and MITCHELL 1952.
† MITCHELL and MITCHELL 1954.
‡ The symbol, v, represents the appropriate visible mutant, *col*, *cot* or *sn*.

percent; *sn*$^+$ *col-4*, 4.2 percent; *sn*$^+$ *col-4*$^+$, 12.8 percent. A few of the auxotrophs (31 percent) were isolated and found to respond to arginine plus lysine. In this connection it is of interest that *sn*, which is of spontaneous origin and has been found as an infrequent segregant in several crosses, was first isolated from a cross involving *col-4*. It seems that *sn* and *col-4*, and possibly *cot*, may be related to one another in a way not elucidated by supposing them to be mutations at three different sites, two linked and one unlinked.

If one considers the crosses of auxotrophs to prototrophs, expected to give 50 percent prototrophs, and crosses between supposedly unlinked mutants, from which are expected 25 percent of progeny showing neither mutant phenotype, one again encounters the numbers which recur as frequencies of recombination of linked mutants. But here they represent deviations from the expected values of 50 or 25 percent. The more frequent deviations are around 2 or 3 and 7 or 8. In the past such discrepancies have been dismissed as possible reflections of differences in viability, since the germination is usually not 100 percent. Yet if one dismissed the 7.6 percent excess of mutants in cross 1 as due to failure of the wild spores to germinate as frequently as the mutants, then, to be consistent, one would need to dismiss the difference between 1.42 per cent *col-4*$^+$ *cot*$^+$ *pyr*$^+$ and 7.2 percent of both *col-4 cot*$^+$ *pyr*$^+$ and *col-4 cot pyr*$^+$ in cross 74. But if this is done, there remains no reason for supposing *col-4*$^+$ *cot*$^+$ *pyr*$^+$ to result from double crossovers and the other two classes of prototrophs from single crossovers; hence the basis for assigning the gene order, *col-4 cot pyr*, then disappears. If the differences in frequency of these three classes are not disregarded, then it may be of interest to attempt to find an explanation of the fact that the 1.72 percent excess of *pyr*$^+$ progeny so nearly parallels the frequency of *col-4*$^+$ *cot*$^+$ *pyr*$^+$ (1.42 percent).

The demonstration of 1:1 segregation by analysis of whole asci has often been taken as evidence of the 1:1 relationship between mutants and their wild counterparts. However, the number of asci examined per cross has usually been small, around 20 to 40, which represents, of course, 160 to 320 spores. Because of the sterility of many of the crosses (to be discussed below) even a small number of complete asci is often not easily found and may represent, therefore, a highly selected sample, giving a limited picture of events in the cross.

Assignment of gene order

Some of the data in Tables 2 and 4, from crosses involving mutants of group IV, have been published more fully along with a map of this group (MITCHELL and MITCHELL 1954). When this map was being prepared there was, in several instances, some uncertainty about the order assigned. To consider first the crosses 75, 76 and 77, if a linear order is given to *ad-6*, *pan*, *cot* and *hist-4*, two of the three nutritional mutants must, of course, be on the same side of *cot*. Hence, one of the three crosses should give a low frequency of either *cot*$^+$ or *cot* prototrophs since one of these would have to arise as double crossovers. It might have been supposed that the *cot* prototrophs from *cot hist* × *pan* (76) were double

crossovers and that the order was *ad cot hist pan*, although if single crossovers in this cross occurred between *cot* and *hist* with about the same frequency as in *cot hist* × *ad* (77) the frequency of double crossovers would have been too high. It was noticed, however, that both *cot hist* × *pan* and *cot hist* × *ad* gave two types of *cot*$^+$ prototrophs, "typical" and "atypical," but that *pan* × *cot ad* gave only the "atypical" type. The atypical *cot*$^+$ prototrophs resembled, in their growth habit on agar plates, the pseudowilds found from other crosses (MITCHELL, PITTENGER and MITCHELL 1952; PITTENGER 1954) although usually with a lower frequency. The pseudowilds had been found to behave as heterocaryons from which both parent mutants of the same mating type could be recovered by isolating single conidia. Samples of the "atypical" wilds were therefore tested and found, indeed, to behave as heterocaryons. It was then supposed that *cot*$^+$ progeny from *pan* × *cot ad* were not products of crossing over and could be disregarded, so that the gene order, *ad pan cot hist*, could be assigned. More recently it has been observed that from cross 3, of wild × *hist-4*, one third of the prototrophs were "atypical" and from wild × *hist-3* (2) about two thirds were "atypical." Cross 19, *cot* × *hist-4*, was then found to give not only at least two types of *cot*$^+$ *hist*$^+$, but also two types of *cot hist*$^+$ progeny, more extreme and less extreme, or "dilute," occurring with very nearly the same frequency. A similar situation was found in *cot* × *hist-3* (18) and *cot* × *hist-1* (17) although in the latter cross the two types of *cot*$^+$ *hist*$^+$ and *cot hist*$^+$ progeny were less sharply defined, as if there were more than two types in each case. These results make it evident that the gene order, *ad pan cot hist*, is not adequately demonstrated.

The obstacles encountered in assigning order to the markers closely linked to *col-4* have been described (MITCHELL and MITCHELL 1954; MITCHELL 1955a,b, 1956). If one class of prototrophs, diagnosed as pseudowilds, was disregarded, the more plausible order appeared to be *pyr-1 pdx col-4 arg-2 pyr-3*, but crosses involving the first three markers gave an unexpectedly high frequency of progeny which would have had to arise as double crossovers or through some mechanism other than crossing over. These progeny were found to behave as if they were genetically pure with respect to the phenotypes they showed and could not, therefore, be dismissed as pseudowilds. Upon finding these recombinants in asci which showed 3:1 segregation of *pdx*$^+$ and *pdx* but 2.2 segregation with respect to the other two markers, *pyr-1* and *col-4*, it was supposed that a mechanism other than crossing over was, indeed, at work. It was assumed that the mechanism involved was analogous to gene conversion, or mutation in heterozygotes (DE SERRES 1956; GILES 1956; ST. LAWRENCE 1956). There appeared to be an association with crossing over, however, since the frequency of the "aberrant recombinants" varied with the frequencies of the "crossover recombinants" in crosses involving different isolates of the mutants.

Prototrophs from *pdx* × *pdxp* (regarded as an allele of *pdx*) were attributed to the same mechanism when they were found in asci which appeared to represent irregular segregations of either *pdx* or *pdxp* because of the absence of the expected *pdx pdxp* segregants from asci in which *pdx*$^+$ *pdxp*$^+$ recombinants were

found. The test for the double mutant, *pdx pdxp*, consisted of backcrossing the phentotypically *pdx* and *pdxp* segregants from these asci to each parent mutant Neither *pdx* nor *pdxp* had been observed to give prototrophs when crossed to itself, hence the double mutant would be expected to give no prototrophs when crossed to either parent. By this test each of the mutant segregants behaved as a single mutant by giving prototrophs when crossed to the parent phenotypically unlike itself.

An attempt to find other cases of "aberrant recombination" has involved some closely linked mutants in linkage group I and has led to ambiguities not only in assigning gene order but also in distinguishing between double and single mutants. A recombinant tetrad from *sn hist-2* × *hist-3* (84) at first appeared entirely regular. By the backcross test (The two *hist* mutants are identical in phenotype (HAAS *et al.* 1952).) it appeared to be of the following constitution:

sn hist-2 hist-3
sn hist-2 hist-3+
sn+ *hist-2*+ *hist-3*
sn+ *hist-2*+ *hist-3*+

With the gene order given above, this result is consistent with the occurrence of a single crossover between *hist-2 and hist-3*. The appearance among random spores of 0.17 percent *sn hist-2*+ *hist-3*+ progeny was disturbing, however, not only because, with this gene order, they would have had to arise as double crossovers or as "aberrant recombinants" but because they appeared with the same frequency as the "aberrant recombinants" from *pdx* × *pdxp*. An essentially identical result was obtained with a recombinant ascus from cross 79 of *sn hist-2* × *hist* (1710), from which 0.06 percent *sn hist-2*+ *hist* (1710)+ segregants were found among random spores.

Recombinant asci from *sn hist-2* × *ad-3* (87) and *sn hist-3* × *ad-3* (91) were, from the standpoint of phenotypes of the segregants, conventional. These phenotypes were as follows:

sn hist-2 ad+	*sn hist-3 ad*+
sn hist-2 ad	*sn hist-3 ad*
and	
sn+ *hist-2*+ *ad*+	*sn*+ *hist-3*+ *ad*
sn+ *hist-2*+ *ad*	*sn*+ *hist-3*+ *ad*+

But not only did the questionable recombinants, *sn hist-2*+ *ad-3*+ and *sn hist-3*+ *ad-3*+, again occur with nearly the same frequencies as in the *pdx* case (0.12 percent and 0.05 percent), the backcross test, as applied in the cross *hist-3* × *sn hist-3 ad-3* (93 and 94) gave results contrary to those expected, namely 21.7 and 17.8 percent prototrophs instead of none; and cross 90, *hist-3* × *sn hist-2 ad-3* appears to give, with respect to *hist-3* and *ad-3*, a different gene order from that found in *sn hist-3* × *ad-3*. From the cross involving all four markers one would suppose *hist-3* to be distal to *ad-3* since *sn*+ *hist-2*+ *hist-3*+ *ad-3*+ is the more frequent prototroph. Three other crosses, intended as checks on the behavior of the *hist-3* strain did nothing to clarify the situation. (The same *hist-3* isolate

was used in crosses 2, 18, 83, 84, 86, 90, 93, 94 and in the cross to *sn* which produced the *sn hist-3* parent of crosses 83 and 91. Random spores from *sn* × *hist-3* were not counted but 23 asci gave an apparently conventional result, consistent with *hist-3* being 7.5 units distal to *sn*). In cross 83 *hist-3* behaved unconventionally by giving 7.9 percent prototrophs when crossed to *sn hist-3*, a descendent of itself; in cross 2 it behaved as a double mutant, giving 67 percent mutant progeny when crossed to wild; and in *cot* × *hist-3* (18) it behaved as a single mutant since 49.9 percent prototrophs were found (Peculiarities of the latter cross have been mentioned.).

It seems, then, that *hist-3* can be regarded as a single-gene mutant assignable to a position in a lineal map only if the results of certain crosses are rejected, but there seems no justification for rejecting these crosses and accepting others. Nor does rejection of *hist-3* on the grounds that it is a "peculiar" mutant seem justified or even very helpful. The behavior of *hist-2* in crosses with *lys-4* and *arg-3* is not easily explained in conventional terms. An attempt to analyze complete asci from *sn hist-2* × *lys-4* (101) had to be abandoned because of the appearance of segregants which, although they germinated, were unable to grow on minimal medium supplemented with lysine and histidine. (Complete medium could not be used since it inhibits growth of *hist-2*.) About one in five asci were found to contain these segregants which, it appeared, could not be *hist lys* double mutants since they were not accompanied, in the same ascus, by *hist*$^+$ *lys*$^+$ segregants. Neither parent showed a requirement other than its own, nor did either behave as a double mutant when crossed to *sn* (30 and 32). Yet it seemed that they could recombine with each other in such a way as to produce an additional requirement. With respect to crosses of *hist-2* and *arg-3* (95, 96, 97, 98 and 99) it may be seen that 98 gave a conflicting result. In 99, in which *sn arg-3 hist-2* is crossed to *sn*$^+$ *arg-3*$^+$ *hist-2*$^+$, the two auxotrophs appear to have recombined much as they did in repulsion (95 and 96) but in 98, *sn arg*$^+$ *hist*$^+$ × *sn*$^+$ *arg hist*, they gave, instead of more than 50 percent auxotrophs as expected, an excess of prototrophs which is nearly the same as the excess of auxotrophs from *sn*$^+$ *arg*$^+$ *hist*$^+$ × *sn arg hist*.

Curiously, quite a similar situation is seen in crosses of the *arg-3 lys-4* double mutants. Crosses of *sn arg lys to sn*$^+$ *arg*$^+$ *lys*$^+$ (104 and 105) gave more than 50 percent auxotrophs as expected, but *sn arg*$^+$ *lys*$^+$ × *sn*$^+$ *arg lys* gave a little more than 50 percent prototrophs. The unpredicted behavior of *sn arg*$^+$ *lys*$^+$ × *sn arg lys* has already been mentioned. This cross not only gave 69 percent prototrophs although it was expected to give less than 50 percent; it gave 16 percent *sn*$^+$ progeny although it was supposedly homozygous with respect to *sn* and, further, 4.2 percent of the progeny were phenotypically indistinguishable from *col-4*, a mutant similar to *sn* but assigned to another linkage group. Another cross (33) in which *sn* was homozygous, involved the *sn arg*$^+$ *lys*$^+$ parent of the above cross (This was the *sn* parent in crosses 28, 29, 30, 32, 98 and 103 also.) and an *sn arg*$^+$ *lys*$^+$ segregant from 102 and gave, not only 1.9 percent *sn*$^+$ progeny, but also 1.6 percent auxotrophs.

Growth responses

A number of cases are known in which nutritional mutants appear to undergo changes in their growth responses as a result of recombination. One of the more carefully examined cases was reported by HASKINS and MITCHELL (1952) and concerned a mutant (39401) which responds to either trytophan or nicotinamide. In certain crosses this mutant showed frequent modifications such that its growth responses became more like those of mutants thought to be at other loci and to control other steps in the biosynthetic pathway concerned. Similar situations found with arginine, lysine and pyrimidine mutants have already been referred to (HOULAHAN and MITCHELL 1948; MITCHELL and MITCHELL 1952). The latter studies culminated in the finding that the original isolate of the pyrimidine mutant involved (37301) could, under certain culture conditions, utilize either lysine or pyrimidine to satisfy its growth requirement. Certain recombinants of this *pyr* mutant with lysine mutants appeared to have acquired a requirement for arginine although an inhibition by arginine had characterized one, or both parents. It has also been observed that crosses of *pyr* to a proline mutant (21863) appeared to give at least two kinds of double mutants, one responding, and the other failing to respond to proline plus pyrimidine. Another case, observed long ago, was that of two thiamin mutants (17084 and 56501) crossed to a third (50005). From these crosses any ascus containing wilds was found also to contain an equivalent number of segregants which did not respond to thiamin. Presumably these were the double mutants but they were not analyzed genetically nor was their requirement determined.

The belated discovery that the *pyr* mutant can utilize lysine suggests the possibility that if more exhaustive growth tests were applied, growth responses of the mutants might, in general, appear less simple and straightforward. The apparent acquisition of new growth requirements calls to mind the fact that the way in which many of the genetic tests have been performed did not take into account the possibility that new requirements might arise. Crosses designed to test for 1:1 segregation of mutant and wild alleles in asci have often been examined by establishing cultures from single ascospores on complete medium and then testing these by subculturing on minimal medium. This, of course, only demonstrates a requirement for something in the complete medium. Crosses between mutants having the same requirement were regularly examined in the same way and, in some cases, when two requirements were involved, the double mutants were identified merely by their failure to respond to either growth factor alone. Thus we do not know to what extent the growth responses reappear unchanged from crosses. It can be said, however, that in the relatively few crosses for which minimal medium with restricted supplements was used to establish cultures, segregants with unexpected requirements were by no means rare. The appearance of such segregants from *hist-2* × *lys-4* (101) has been mentioned above, as has the *sn* × *sn* cross (33) which gave 1.6 percent auxotrophs. Cross 32, *sn* × *lys-4* gave 1.2 percent auxotrophs among 1027 spores plated on minimal plus lysine. From cross 91, *sn hist-3* × *ad-3* about one ascus in ten contained seg-

regants which did not grow on the combined supplement of adenine and histidine. When random spores were plated on this medium 4.1 percent among 552 failed to grow. Cross 48, *hist-4*○× *hist* (1710) gave 1.9 percent auxotrophs among 940 spores on minimal plus histidine. Among nine asci from cross 38, *ad-6* × *tryp-1* (which gave 45 percent prototrophs among random spores) three were found to contain segregants which grew very little on minimal plus adenine and trytophan but grew well on complete. Curiously, these segregants were albino on the minimal medium but normally colored on complete. Their requirement appeared partially satisfied by adenine plus methionine but the albino character was expressed on this medium, too.

Sterility

It is well known that very many of the Neurospora crosses are characterized by frequent ascus and spore abortion. A cross from which as many as 20 percent of the asci contain eight normal-appearing spores is considered rather fertile. The sterility is not usually traceable to any simple cause. More often it appears and disappears in an unpredictable fashion, even in crosses involving closely related strains. A fairly general approach to this difficulty is that if one can get some spores or asci to examine, one examines them, without undue concern about those that failed to appear.

Recently an attempt has been made to recheck some of the linkage map results by starting again with some highly fertile strains which had not been intercrossed. For this purpose the original isolates of several mutants were obtained from conidia lyophilized in 1944, soon after the mutants were isolated (BEADLE and TATUM 1945). These included *pyr-3a* (37301), *pyr-3b* (37815) and *lys* (37811), derived from the same wild parents, one of which (25a) was also obtainable from an old lyophilized culture. The results of crosses with these strains were disappointing. No highly fertile crosses were obtained. That of *pyr-3a* to *lys* was completely sterile and the others gave around 20 percent of asci with normal-appearing spores but perhaps as many as five percent had only four spores. This had not been noticed previously.

The four-spored asci were of at least two types, one long enough to have contained eight spores and the other just long enough for four. Germination of spores from these asci was poor but in two from Wild 1A × *lys* there was 1:1 segregation of *lys* and *lys*$^+$. A cross of one of the *lys* segregants to a wild from a four-spored ascus of Wild 1A × Wild 25a seemed somewhat less fertile than either parent cross and still gave both four- and eight-spored asci.

The cross of *pyr-3b* × *pyr-3a* had previously been found to give 1:1 segregation in complete asci (HOULAHAN, BEADLE and CALHOUN 1949), but when random spores were now examined at 25° C (Mutant *pyr-3b* is temperature-sensitive and not fully expressed at 25° C) only 25 percent among 1050 spores were classifiable as *pyr-3a*.

The cross more thoroughly examined was that of the original isolate of *inost* (83201, derived from Wild Abbott 4A × 25a), also temperature-sensitive, to *pyr-3a*. At 35° C it gave 34.4 percent prototrophs instead of the expected value of

25 percent (53) and at 25° C, only 28 percent of progeny classifiable as *pyr-3a* instead of 50 percent as expected. Asci with eight mature spores (about 10 to 20 percent) appeared fairly regularly to show 1:1 segregation of two spore sizes. Of 33 asci dissected only one germinated completely. Segregation in this one was as follows:

pair 1	*inos*$^+$	*pyr*$^+$
pair 2	*inos*$^+$	*pyr*$^+$
pair 3	*inos*	*pyr*
pair 4	*inos*	*pyr*

Four crosses of these segregants showed no increased fertility, 1 × 3 and 1 × 4 being less fertile than the parent cross. Among spores incubated at 25° C three types could be classified, one presumably representing *inos*$^+$ *pyr* and *inos pyr;* the other two types were prototrophs, one slow-growing and the other "typical," perhaps representing *inos pyr*$^+$ and *inos*$^+$ *pyr*$^+$. The proportions of the three types are given in Table 5. It is unlikely that these frequencies represent chance deviations from the expected 50, 25 and 25 percent. Nor does the idea that the different phenotypes differ in their ability to germinate satisfactorily explain the deviations.

TABLE 5

Crosses of inost *(83201) and* pyr-3a *incubated at 25° C. Parent cross* = inos pyr$^+$ × inos$^+$ pyr; *intraascus crosses* = inos$^+$ pyr$^+$ × inos pyr

	Types of progeny (percent)			Number of spores
	inos$^+$ *pyr*$^+$	*inos pyr*$^+$	*inos*$^+$ *pyr* *inos pyr*	
Expected	25	25	50	
Observed				
Parent cross	36.0	35.7	28.3	294
Pair 1 × pair 3	34.1	29.8	36.0	211
Pair 1 × pair 4	37.4	29.3	33.3	392
Pair 2 × pair 3	29.2	27.2	43.6	1223
Pair 2 × pair 4	30.9	28.2	40.9	932

The further observation was made that, from the parent cross, among six asci in which at least one member of each spore pair germinated two showed irregular segregations as follows:

inos$^+$	*pyr*$^+$	*inos*	*pyr*$^+$
inos$^+$	*pyr*$^+$	*inos*	*pyr*$^+$
inos$^+$	*pyr*	*inos*$^+$	*pyr*
inos	*pyr*$^+$	*inos*	*pyr*$^+$

Although particular care was taken in the dissections, the possibility that the order was incorrectly recorded is not ruled out, since, in the first ascus listed only one member of each spore pair germinated and in the second, only one member of pairs 1 and 2.

Reverse mutation and somatic segregation

When nutritional mutants appear to have reverted in vegetative culture it has sometimes been possible to find, from outcrosses of the reverted strains to standard wild, frequent asci in which all segregants are prototrophs, as one would expect if reverse mutation had occurred. In other cases there are found asci from which more than four spores give rise to prototrophs but the segregations are those expected if mutation to a suppressor of the mutant character had taken place. Sometimes, however, the presence of wild nuclei in a reverted strain has been difficult to demonstrate.

The latter situation was found with reverted *pdx* (37803) and *pdxp* (39106) segregants from crosses between these two mutants (MITCHELL 1956). A rather high incidence of reversion was found if conidia from the mutant segregants were inoculated into minimal medium and left standing for several weeks. When the reverted isolates were backcrossed to the same mutant, unreverted, no prototrophs were found among 5,000 to 10,000 offspring even though the protoperithecia had been formed by the reverted strain on minimal medium. Prototrophs were obtained from such backcrosses only after repeated subculturing of the reverted strain on minimal medium. Yet if conidia from the original reverted cultures were plated on minimal medium many of them were able to grow. A similar situation had been found earlier with revertants of *thi-2* (9185) (unpublished data of DR. G. W. BEADLE and the author).

It now seems that an interpretation of these cases, not in terms of reverse mutation, but of somatic segregation may be more fitting. It seems that, through somatic segregation, nuclei may arise with a chromosome complement such that they confer upon a strain, pure, with respect to them, the capacity for independent growth. These nuclei might reappear from an outcross or a backcross. If, on the other hand, independent growth depends upon the interaction, in the resulting heterocaryon, of the new nuclei with the old, prototrophs might not reappear from backcrosses. The prototrophs found after repeated selection might be due to a secondary process.

DISCUSSION

It is, of course, difficult to consider, at this time, the possibilities that we have not demonstrated the nutritional mutants of Neurospora to be due to "single-gene changes" and that we have not been able to "locate" them satisfactorily on "linear maps." Yet it is hardly possible not to see that the inconsistencies and ambiguities as well as the unpredicted regularities observed in genetic behavior may have a common explanation, merely that the nuclear behavior in developing asci and the number of chromosomes in the complement are variable. If it is supposed that expression of the mutant characters is determined in a manner analogous to that in which sex is thought to be determined in Drosophila, that is, by the relative dosages of certain chromosomes, then it seems that flexibility sufficient to account for the actual observations may exist, particularly if similar but slightly different chromosomes have been introduced into the stocks from different wild sources. As MCCLINTOCK (1954) has already suggested, it seems futile to attempt a serious,

detailed interpretation of any cross without knowledge of the nuclear behavior in *surviving* asci. The recurrence of certain prototroph frequencies suggests that there are in general certain patterns of nuclear behavior which lead to the formation of viable spores. Possibly a more precise analysis, both genetically and cytologically, of fertile crosses will reveal the nature of some of these patterns.

With regard to the growth requirements, it already appears likely that relationships between mutants having different requirements as well as between those having like requirements have been misunderstood. Perhaps a more thorough analysis, from a point of view which allows as much significance to be attached to the "modifiers" as to the "primary characters," may be revealing here too.

SUMMARY

1. Recurrence of certain frequencies of recombination of "linked" mutants was observed by classifying and counting random ascospores about 12 hours after germination. The more commonly recurring frequencies of various classes of prototrophs are between 0.5 and three percent and around seven and eight percent. These same numbers appear as frequent deviations from expected frequencies of 25 or 50 percent in crosses of "unlinked" mutants or of mutants to wild.

2. In several "map regions" analysis of "closely linked" mutants failed to produce convincing evidence of a "linear order" of the markers.

3. Results of backcross tests used to classify mutant segregants as "single" or "double" mutants suggest that this distinction is meaningless except in application to specific crosses. The same mutant isolate may give the result expected of a "double" mutant in one cross but behave as a "single" mutant in another.

4. It appears that in crosses of nutritional mutants the growth responses may undergo frequent changes and new requirements may arise as a result of recombination.

An expansion and revision of basic assumptions to make them more applicable to the actual observations seems indicated. It is tentatively suggested that if the phenotypic expressions are regarded as reflections of differences in dosages of the chromosomes, a more realistic picture may be obtained through detailed cytological and genetic studies.

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BLOOD GROUPS IN SHEEP. I. THE X-Z SYSTEM[1]

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PREVIOUS studies of blood groups in sheep, including those of ANDERSEN (1938), RENDEL (1957) and SPRAGUE (1957), have been primarily concerned with the serology and genetics of the R-O system, and with its relationship to the A-B-O system of man, the J system of cattle, and the A-O system of swine. Several investigators (ANDERSEN 1938; YCAS 1949; and DUJARRIC DE LA RIVIÈRE, MILLOT and EYQUEM (1952) have reported that immunizations with sheep erythrocytes may give rise to hemolysins which distinguish differences in sheep bloods other than those seen in R-O typing, but there have been no reports of definitive systems other than R-O. It is the purpose of this report to present evidence for a second system of ovine blood groups, the X-Z system.

MATERIALS AND METHODS

The reagents used in typing for X and Z were developed from heteroimmune antisera which were prepared by immunization of rabbits with erythrocytes of sheep as well as other species in the families Bovidae and Antelocapridae. Certain of the rabbit antisera used in the present study were the same as those prepared and used by STORMONT and SUZUKI (1958). When more than one blood was used to immunize any one rabbit, equal amounts of blood from each donor were pooled. During a period of three weeks, nine intravenous injections were made (on alternate days) of 0.5 ml whole blood or the equivalent in erythrocytes suspended in isotonic saline (0.91 percent sodium chloride solution). The antisera were collected four to seven days after the last injection, and were kept at −20° C when not in use.

The antisera were absorbed with erythrocytes of individual sheep to determine whether they contained specific fractions of antibodies defining individual differences in sheep. Blood for the absorptions was obtained from an experimental flock of 40 sheep. The sheep were kept at the Armstrong Tract of the University of California at Davis, for use in studies of blood groups. They were bled by puncture of the jugular vein, and the blood was collected in 250 ml evacuated bottles containing 100 ml Alsever's solution (2.05 percent dextrose, 0.8 percent sodium citrate and 0.42 percent sodium chloride adjusted to a pH of 6.2 with ten percent citric acid). Samples of the antisera were diluted ⅛ with isotonic saline

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and heated at 56° C for 30 minutes before absorption. Ordinarily, three successive absorptions (10 min, 30 min, and 30 min) were required for complete removal of antibodies for the absorbing cells when a ratio per absorption of 0.75:1.0 of washed, packed erythrocytes to diluted serum was used. The decanted serum from the third absorption was kept at −20° C if it was not used immediately, and was heated for 15 minutes at 56° C, just prior to testing.

In addition to the blood from the experimental flock, citrated blood samples were obtained for genetic studies from the flock of purebred Shropshires of Mrs. Ida M. Groverman and Son at Petaluma, California, from the flocks at the University of California at Davis which included Suffolks, Hampshires, Rambouillets, Corriedales and Targhees, and from Targhees and Rambouillets at the U. S. Sheep Experiment Station at Dubois, Idaho. The samples were collected in 15 ml tubes containing 3 ml isotonic citrate solution (2.0 gm sodium citrate plus 0.5 gm sodium chloride per 100 ml). The blood samples were refrigerated until used.

The bloods were tested in a hemolytic test. The reaction of antigen (red blood cells) and antibody (antiserum) in the presence of complement was observed. The complement used was either fresh guinea pig serum diluted 1/15 to 1/20 or undiluted absorbed fresh rabbit serum. Often duplicate tests were set up, one with guinea pig and one with rabbit complement. The rabbit complement was absorbed with washed packed erythrocytes from several sheep to remove natural heterohemolysins for sheep red cells. Two absorptions of ten minutes each at refrigerator temperatures, and centrifugation in a refrigerated centrifuge were adequate to remove normal antibodies, using a ratio of cells to serum of 1:4 per absorption.

The blood typing tests were set up as follows: to a 10 × 75 mm tube containing 0.10 ml of the diluted antiserum, 0.05 ml of a two to three percent suspension of washed erythrocytes was added. The tubes containing antiserum and red cells were shaken and allowed to stand a few minutes before the addition of 0.05 ml of complement. The test was read at one half hour, when slight (tr), incomplete (±) and complete (+) hemolysis was recorded. At 2½ to 3 hours and 4½ to 5 hours, degrees of hemolysis from 0 (no hemolysis), 0+, tr, 1, 2, 3 and 4 (complete hemolysis) were recorded. All tests were performed at room temperature (24° ± 3° C) and the tubes were shaken after the first two readings.

EXPERIMENTAL RESULTS

Preparation of anti-X

Tests of absorptions of an antiserum produced in a rabbit (X3) against the erythrocytes of eight Karakul sheep showed that absorption with the red cells of any one of nine out of ten sheep exhausted all of the hemolysins (Table 1).

Absorption with red cells of No. 1457 failed to remove hemolysins for the red cells of seven of the nine sheep whose red cells seemed to exhaust all the antibodies. Since the erythrocytes of sheep No. 572 and No. 668 absorbed all the antibodies but were nevertheless not lysed by antibodies remaining in the serum following absorption with No. 1457, it seemed probable that dosage reactions

TABLE 1

Tests of absorptions of Karakul (X3) antiserum

Test cells	Absorbing cells										
	559	572	595	630	668	999	1457	2055	2083	2355	Rabbit complement control
559	0	0	0	0	0	0	3	0	0	0	0
572	0	0	0	0	0	0	0	0	0	0	0
595	0	0	0	0	0	0	4	0	0	0	0
630	0	0	0	0	0	0	4	0	0	0	0
668	0	0	0	0	0	0	0	0	0	0	0
999	0	0	0	0	0	0	4	0	0	0	0
1457	0	0	0	0	0	0	0	0	0	0	0
2055	0	0	0	0	0	0	4	0	0	0	0
2083	0	0	0	0	0	0	1	0	0	0	0
2355	0	0	0	0	0	0	3	0	0	0	0

were involved. The antibodies remaning in the serum following absorption with the red cells of No. 1457 might be of proper quantity and quality to lyse erythrocytes from individuals homozygous for a blood factor X, but not those heterozygous for this factor. This anti-X serum stimulated a search for a complementary blood factor, and the discovery of such a blood factor, designated Z, made it easier to study systematically the possible dosage reactions.

Preparation of anti-Z

Anti-Z was discovered in several antisera prepared in rabbits against erythrocytes of individual species, including springbok, blesbok, eland, pronghorn antelope and bison. These antisera were individually absorbed with erythrocytes of two type-X sheep. Selected antisera produced in rabbits against red cells of these species showed rather marked hemolytic activity for erythrocytes of sheep No. 1457, as well as some hemolytic activity for erythrocytes of sheep No. 572 and No. 673 (Table 2).

An antispringbok serum seemed most promising as a source of antibodies against Z, and by individual absorptions of this serum, an appropriate absorbing cell of type X was selected for preparation of a Z reagent.

Effects of dosage and complement

Reactions of the original X and Z reagents were compared in parallel tests on the bloods of the 40 sheep in the experimental flock, and three blood types were defined, as follows: 29 were type X, ten were type XZ, and one was type Z. Twenty rabbits were immunized with erythrocytes of sheep No. 1457, the only type-Z sheep in the flock, and usable Z reagents were prepared from 14 of these antisera. Of 14 rabbits immunized with erythrocytes of type-X sheep, two produced antisera from which usable X reagents were prepared. Over 1000 sheep were tested with X and Z reagents and the existence of three types, X, XZ and Z was confirmed. Not one of the bloods was negative for both X and Z.

TABLE 2

Summary of tests of absorptions of antisera containing anti-Z

Absorbing cells: Test cells	Antisera produced in rabbits against red cells of Bovidae: Eland (10P) 595	Eland (10P) 630	Blesbok (W) 595	Blesbok (W) 630	Springbok (B) 595	Springbok (B) 630	Bison (X32) 595	Bison (X32) 630	Antelocapridae: Pronghorn (X4) 595	Pronghorn (X4) 630	Guinea pig complement control
595	0	0	0	0	0	0	0	0	0	0	0
630	0	0	0	0	0	0	0	0	0	0	0
511	0	0	0	0	0	0	0	0	0	0	0
559	0	0	0	0	0	0	0	0	0	0	0
572	2	tr	1	1	3	4	tr	tr	tr	tr	0
634	0	0	0	0	0	0	0	0	0	0	0
673	1	tr	1	1	4	4	tr	tr	tr	tr	0
999	0	0	0	0	0	0	0	0	0	0	0
1457	2	2	4*	4*	4*	4*	2	2	4*	4*	0
2355	0	0	0	0	0	0	0	0	0	0	0

* Complete hemolysis in ½ hour.

The Z reagents consistently lysed type-Z erythrocytes much more rapidly than those of type XZ. The type of complement used in the test was important in contrasting erythrocytes of type X and type XZ. When guinea pig complement was used, type-Z cells were rapidly and completely lysed by the Z reagents, whereas type-XZ cells were lysed more slowly, and the hemolysis was rarely complete at the end of five hours. When rabbit complement was used, type-Z cells were usually lysed as rapidly as if guinea pig complement was used, but the type-XZ cells were often not lysed at all; if there was any hemolysis, it was slight and took place slowly. Z reagents from more than 20 different antisera were studied, and although this difference between rabbit complement and guinea pig complement was more marked with some of them than with others, it was always apparent. In order to clearly differentiate cells of type XZ and Z by the use of Z reagents, each potential reagent was titrated and a dilution of the antiserum was chosen which most clearly differentiated between cells of the two types. The antiserum was then used, at the dilution chosen, as a dosage reagent.

Similarly, the dilution at which the X reagents most clearly differentiated cells of type X and XZ were determined, for the preparation of dosage reagents. As a general rule, the X reagents lysed type-X and XZ cells with equal rapidity when guinea pig complement was used in the test. When rabbit complement was used, almost no lysis was observed in tests with one of the X reagents, but two others prepared from two different antisera lysed most type-X erythrocytes more rapidly than most cells of type XZ. However, the degree of hemolysis varied considerably in different families of sheep, and although these X reagents made it possible in most cases to distinguish between X and XZ erythrocytes from sheep of the same family, it was not dependable for this purpose when erythrocytes of more than one family were tested in the same test. Thus if cells of type X from one family were lysed as slowly as the cells of type XZ from another family, these cells of

type X and type XZ could not be differentiated, even though cells of type X and type XZ within one family were lysed at different rates.

One striking exception to the dosage effects was seen in the tests of the erythrocytes from one of the sheep in the experimental flock. The cells of sheep No. 577 were negative when tested with the X reagent when rabbit complement was used, but were type X rather than type XZ.

Genetic analysis

The serological evidence strongly suggested that X and Z were acting as a pair of complementary blood factors, and that two alleles, X and X^z, controlled the expression of the three phenotypes **X**, **XZ** and **Z**. The distribution of these phenotypes among 40 sheep in our experimental flock checked exactly with genetic expectations based on the Hardy-Weinberg Law: 29 were type X, ten were type XZ and one was type Z. These 40 sheep were of a variety of breeds, pure and mixed, and might be considered a representative sample of California sheep, although certainly not a random sample.

Additional data are given in Table 3. The "white-faced" breeds included Corriedales, Targhees and Rambouillets, as well as some Rambouillet-Merino crossbreds. It was only in the white-faced breeds that both alleles appeared to be segregating and the distribution of the three phenotypes in this population was in agreement with expectation. The allele X was completely fixed in the "black-faced" breeds studied (primarily Shropshires and Suffolks).

TABLE 3

Frequency of R, O, X, XZ and Z in sheep of white-faced breeds

Blood type	X	XZ	Z	Totals
R	112	79	12	203
O	102	51	6	159
Totals	214	130	18	362

Chi-square of test for independence of X-Z and R-O = 3.23
d. f. = 2 $p > 0.05$

Most of the blood samples which were typed for X and Z were also typed with R and O reagents in stock in this laboratory, and no association of blood factor X or Z with R or O was noted, as is shown in Table 3, which summarizes tests for R, O, X and Z in white-faced breeds, where all of the expected types RX, RXZ, RZ, OX, OXZ and OZ were found.

The gene responsible for production of the **R** phenotype is dominant to that for **O** (STORMONT 1951; RENDEL, NEIMANN-SØRENSEN and IRWIN 1954). The symbol R is used for the gene responsible for the blood type R, and r^o for the gene which when homozygous ($r^o r^o$) results in blood type O. The offspring of a ram of genotype $Rr^o XX^z$ and ewes of genotype $r^o r^o XX$ were of all four types expected in progeny from a backcross, and the numbers of each type observed were: one

Rr^o XX, two Rr^o XX^z, two r^or^o XX and two r^or^o XX^z. These data exclude the possibility that the genes responsible for the six phenotypes **RX**, **RXZ**, **RZ**, **OX**, **OXZ** and **OZ** are members of the same allelic series.

Because of the relative rarity of type-Z sheep in the families studied, most of the data involved matings of type-X and XZ sheep. Results of tests on the bloods of 259 lambs, their dams and sires are shown in Table 4. These data are in accord with the hypothesis of a two-allele system.

TABLE 4

Inheritance of X-Z blood groups in 259 lambs

Kind of mating	Number of offspring of type		
	X(genotype XX)	XZ(genotype XX^z)	Z(genotype X^zX^z)
$XX \times XX$	172	0	0
$XX \times XX^z$	24	34	0
$XX \times X^zX^z$	0	4	0
$XX^z \times XX^z$	4	12	7
$XX^z \times X^zX^z$	0	1	1

DISCUSSION

The X-Z system of sheep resembles the F-V system of cattle described by STORMONT (1952). In both of these systems, only a single pair of alleles appears to be involved at present, and in both systems dosage reactions were observed and were of initial importance in the elucidation of each system. In neither were they necessary for complete genotypic and phenotypic classification if reagents for both F and V or X and Z were available. In the F-V system, dosage reactions were observed with most F reagents produced from rabbit antisera, but not with V reagents. In the X-Z system, dosage reactions were observed with both anti-X and anti-Z.

In discussing the F-V system, STORMONT (1952) stated that the usual explanation for these differences in sensitivity of the blood cells was that two doses of the gene produced more of the same antigen than did one dose. He also cited OWEN's (1948) work as evidence for demonstrating the principle of competition of alleles or their products for the same substrates. The differences in reactivity of cells from homozygotes and heterozygotes for the alleles X and X^z may be explained on the basis of either of the above hypotheses, when a reagent for X or Z is tested with a single type of complement and dosage reactions are observed. However, the two X reagents described in this report showed dosage effects with rabbit complement, whereas cells of type X and type XZ were indistinguishable when guinea pig complement was used. If a difference in the amount of antigen produced in heterozygotes and homozygotes were the sole basis for the dosage effect, it might be expected that it would be just as apparent with guinea pig complement.

The most pronounced differences in rates and degrees of hemolysis were usually observed when the reagents were quite dilute. When less dilute, the reagents

lysed erythrocytes from both heterozygotes and homozygotes more rapidly, and differences were obscured. However, the differences between the tests with X reagents when different sources of complement were used were maintained regardless of how greatly the reagent was diluted. Similarly, the dosage reactions with the Z reagents were more marked when rabbit complement was used, regardless of the dilution of the antiserum.

Although at present only two alleles are known to be involved in the X-Z system, occasional cells of type X (such as those of sheep No. 577 described above) failed to react with the dosage reagent when used with rabbit complement and were more slowly lysed by the X reagents used in this study than were other type-X cells when used with guinea pig complement. Such reactions suggest that subtypes of X may exist, or that other reagents might be developed which could detect new phenotypes in the X-Z system.

As already pointed out, no XZ or Z phenotypes were observed in sheep of the black-faced breeds. More than 200 sheep, primarily Shropshires and Suffolks, were tested. In data for 362 sheep of white-faced breeds (Table 3, which includes Corriedales, Rambouillets and Rambouillet-Merino crossbreeds) the frequency of the allele X^z was 0.23. The greatest deviation in frequency of this allele from the average frequency was observed in small lines of Rambouillets, where it varied from approximately 0.5 to 0.0. The frequency of the allele X^z was close to 0 in three small families, and close to 0.5 in two. No typing for X and Z had been done previously, but these data suggested that as expected, allele X^z may be lost or may be increased in frequency in small families, and repeated testing for X and Z in flocks in which the alleles X and X^z are segregating would help, in conjunction with other markers, to measure progress in inbreeding.

SUMMARY

1. Evidence is presented for a second system of ovine blood groups, the X-Z system, which identifies a genetic locus different from the locus for the R-O system.

2. The X-Z system comprises three phenotypes, **X**, **XZ** and **Z**, which appear to be controlled by a pair of alleles, X and X^z. These phenotypes are detected by means of reagents prepared from antisera produced in rabbits against erythrocytes of sheep as well as certain related species in the families Bovidae and Antelocapridae.

3. The Z reagents differentiate bloods of type XZ from those of type Z by means of differences in rates and degrees of hemolysis. In the hemolytic test erythrocytes of homozygotes (type Z) are rapidly and completely lysed whereas those of heterozygotes (type XZ) are more slowly and less completely lysed by Z reagents. These differences are more pronounced if rabbit complement rather than guinea pig complement is used in the test.

4. The X reagents may be used to differentiate bloods of type X from those of type XZ, but the dosage effect is much less pronounced than that obtained with Z reagents, and is apparent only when rabbit complement is used.

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LINKAGE STUDIES IN GOSSYPIUM. I. ALTERED RECOMBINATION IN ALLOTETRAPLOID G. HIRSUTUM L. FOLLOWING LINKAGE GROUP TRANSFERENCE FROM RELATED DIPLOID SPECIES

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SPECIATION among Gossypium species has been suggested to result by multiple gene substitution and mutation as well as by cryptic structural differentiation of the chromosomes (STEPHENS 1950). Hybrids between species may show no cytological evidence of gross chromosomal divergence, but genetic recombination between linked loci in these hybrids might be expected to differ from that of the parental species due to cryptic structural differentiation of the chromosomes carrying the linked factors. Such a change in recombination has been reported by LODEN (1950) for the red plant-cluster (*R1-cl1*) linkage in *G. hirsutum* × *G. barbadense L.* This change was attributed to cryptic structural differentiation of the chromosomes in the hybrid.

On the other hand, a reduction in genetic recombination of interspecific hybrids could result from genes having a specific effect on chromosome pairing, particularly those causing a lowered chiasma frequency at metaphase. MENZEL and BROWN (1955) report F_2 segregation of plants having lowered chiasmata frequencies from an F_1 *G. hirsutum* × *G. barbadense* cross with normal bivalent pairing. Also, the genetic background or strain genotype will differ in the interspecific as compared with the intraspecific hybrids, which might be expected to alter genetic recombination. This so-called modifer concept is found throughout the cotton genetic literature, it being likely that speciation among Gossypium species has resulted by multiple gene substitution and mutation as well as by cryptic structural differentiation of the chromosomes.

The objective of this paper is to report differences in recombination percentages following linkage group transference from diploid species to allotetraploid *G. hirsutum*. Donor diploid species showing essentially bivalent pairing and normal chiasmata frequency in their interspecific hybrids with one genome of *G. hirsutum* were selected as sources of linkage groups. A decrease in genetic recombination and the resulting inheritance as blocks of genes will be shown to support STEPHENS' (1950) test for speciation by chromosomal structural differentiation. A change in chiasma position and/or frequency will be discussed as the mechanism responsible for this altered recombination percentage.

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MATERIALS AND METHODS

Inasmuch as a change in genetic recombination of an interspecific hybrid can be attributed to both chromosomal difference and genotypic influence, the present study was designed to compare the relative importance of chromosome cryptic differentiation to genotypic influence. Interspecific hybrids therefore were actually used not as the ultimate experimental material, but rather as sources of donor species linkage groups. To evaluate any genotypic influence on genetic recombination, a domestic *G. hirsutum* linkage group was transferred to a number of inter- and intra- specific backgrounds. To assess the extra-genic effect (i.e., chromosome structure) on recombination the various diploid linkages were transferred through interspecific hybrids to a common genotypic background essentially that of *G. hirsutum*. Whenever feasible the domestic linkage was studied on a similar genotypic background. Studies were made in first, second, third, and later backcross generations, since the earlier backcrosses were sometimes subject to such difficulties as "photoperiodic" flowering responses and duplicate factor inheritance, (see RHYNE 1957). The data here reported represents segregating progenies scored during the spring and summer of 1956 in North Carolina. The heterozygous generation was grown the preceding winter in Iguala, Mexico. These data collected in 1956 proved to be typical of the information accumulated on earlier generations.

Two linkage groups were particularly useful, namely the duplicated linkage groups of the A_h and D_h genomes of *G. hirsutum* reported by RHYNE (1957). The D_h linkage carried *Cl1* (not cluster fruiting habit), *R1* (incompletely dominant for red plant parts), *Yg1* (duplicate for not yellow-green cotyledons and leaves). The A_h linkage carried *R2* (petal spot, and duplicate locus of *R1* dependent on *R2* allele used), *Yg2* (duplicate for not yellow-green cotyledons and leaves), *Lc1* (brown lint), and *Cl2* (formally written duplicate "normal" allele for the fruiting habit of *G. hirsutum*, the genetics of *Cl2* relative to *Cl1* is poorly understood. This allele was, therefore, only occasionally useful in this study).

The diploid A_2 genome of *G. arboreum* L. was available through the amphidiploid $2(A_2D_1)$ which hybridizes readily with allotetraploid *G. hirsutum* $2(A_hD_h)$ and *G. barbadense* $2(A_hD_h)$. One complex translocation and one ring of four are characteristic of $F_1A_hD_h \times A_2$ interspecific hybrids, the remaining chromosomes form bivalents (except for the univalent D_h chromosomes). The D_1 genome of *G. thurberi* Tod. was available from the same amphidiploid hybrids. These are 13 bivalent chromosomes in $F_1A_hD_h \times D_1$ interspecific hybrids (except for the A_h univalents). The D_5 genome of *G. raimondii* Ulb. was available from the trispecies hybrid $A_hA_2D_hD_5$ and hexaploid $2(A_hD_hD_5)$. Thirteen bivalents are formed in $F_1A_hD_h \times D_5$ hybrids (except for the A_h univalents).

EXPERIMENTAL RESULTS

Recombination in the Cl1-r1-Yg1 *linkage for D_h/D_h genome hybrids*

The D_h linkage carries the linked loci *Cl1-r1-Yg1-dw*, each locus having mutant alleles except dw. Recombination thus was studied for the regions *Cl1-r1* and

r1-Yg1 in a range of genetically diverse genotypes. The experimental materials were constructed by transferring the *Cl1-r1-Yg1* linkage from a *G. hirsutum* doubled haploid (Z106, which also carried *Yg2*, the duplicate of *Yg1*) to several yellow-green varieties (*Cl1-r1-yg1, r2 yg2*). Each of these genetically diverse varieties were then crossed to a *cl1-R1-yg1, r2-yg2*, type. The resulting heterozygote was grown in Mexico during the winter of 1955-56. The last mentioned yellow-green parental type grew poorly in Mexico making backcross progeny testing impossible.

TABLE 1

Recombination at r1-Yg1 *among strains of* G. hirsutum *in* F_2 *progenies, coupling phase* r1-Yg1/R1-yg1 F_1*

Strain (Substrain)	Phenotypic segregation					Recombination among substrains percent
	r-Yg- parent	r-ygyg	RR Yg-	RR ygyg parent	Recombination percent	
31A F_2 (2,4,5)	560	61	49	113	16.48	20.21, 14.42, 14.20
30A F_2 (4,6)	196	32	26	34	25.99	23.23, 28.55
30B F_2 (3)	358	50	35	74	18.86	....
G. hirsutum Total	1114	143	110	221†	18.57†	
Major Gene Segregation		*Yg:ygyg*		P = 0.05		
		r:RR		P = 0.01		
		Linkage		P = 0.01–0.001		

* Actually dominance is incomplete so that three phenotypes, rr, rR, RR, occur. The Rr has been lumped with rr to take advantage of F_2 coupling efficiency.
† Substrains and strains were homogeneous. All strains can be considered a part of the *G. hirsutum* total population having 18.5 percent recombination.

The F_2 recombination data for the *r1-Yg1* region is summarized in Table 1 for three distinct strain (variety) genotypes. The substrains of strain 31A had 16.48 percent recombination using MURTY's (1954) formula for minimum discrepancy in F_2 progeny; Strain 30B had 18.86 percent and 30A gave a value of 25.99 percent. A chi-square test of heterogeneity indicated that the three strains could be considered a part of a homogeneous *G. hirsutum* population having 18.57 percent recombination.

Some loss of parental type *RR ygyg* plants was observed during the early cultivations. The chi-square analysis of the major gene segregations showed a P value of 0.05 for *Yg:yg* segregation and a P of 0.01 for the *r:R* segregation. These poor fits to the expected 3:1 ratio were considered to reflect the cultural conditions rather than disturbed major gene segregation.

Table 2 contains backcross segregations obtained by using SM4, a *Cl1-r1-yg1, r2-yg2* genotype, which produced a few flowers in Mexico. The two substrains of 30A, having only *Yg1*, gave 16.2 percent recombination. The four substrains of 30A, having both *Yg2* and *Yg1*, showed the expected duplicate factor segregation, i.e., 3 *Yg:1yg* and a best fitting recombination value according to chi-square ranging between 16.2 and 20.0 percent. (When duplicate *Yg* alleles are present

TABLE 2

Recombination at r1-Yg1 *among strains of* G. hirsutum *in backcross progenies, coupling phase* r1-Yg1/R1-yg1 F_1

Strain (Substrain	Phenotypic segregation: rr Ygyg parent	rr ygyg	Rr Ygyg	Rr ygyg parent	Recombination percent	Recombination among substrains percent
30A BC(4 and 6)	81	12	14	53	16.2	10.4, 19.3
	Yg2 also segregating, giving duplicate factor ratio					
30A BC(1,2,5,7)	130	16	77	46	16.2–20	16.0–20.0
	Yg2 segregating, Yg1 absent*					
31B1 and 28A7	40	32	44	28	50	.....

Major Gene segregation,	30A BC(4 and 6)	*yg:ygyg*	P = 0.02 linkage 0.01–0.001
		r :RR	P = 0.05
	30A BC(1,2,5,7)	*Yg:ygyg*	P = 0.50 linkage 0.01–0.001
		r :RR	P = 0.10
	31B and 28A*	*Yg:ygyg*	P = 0.02 linkage n.s.

* Note the deficit of ygyg phenotypes that reflects loss due to selection pressure against ygyg plants, loss herein not complicated by linkage.

in a hybrid an inefficient estimate of the recombination usually results, see RHYNE 1957). Again there was an observed loss of parental type plants. This loss is illustrated by the segregation in the two substrains listed at the bottom of the table. Here no linkage of *Yg2* and *R1* alters the expected numbers in the phenotypic classes, so the deficiency in yellow-green phenotypes reflects loss due to cultivation.

F_2 recombination within the *Cl1-r1* region is shown in Table 3 for three strains; strain 30A had an intra*hirsutum* genotypic background while both 28A and 6-26B5 were interspecific genotypes. The recombination for each strain was shown by a chi-square test of heterogeneity to fit the 15.43 percent value for the total population, i.e., there was no evidence of altered recombination within the *Cl1-r1* region due to genotypic background influence. Recombinations values similar to these were obtained for the D_h/D_h linkage in first, second, and third interspecific backcross background.

Major gene segregations are given in the lower half of Table 3. A deficiency of cluster (*clcl*) plants is indicated by the P value of 0.01. The deficiency was most striking in the intra*hirsutum* 30A, presumably because a number of parental type *RR ygyg* plants were not scored for cluster. These were not scored because the season was shortened by an infestation of boll weevil which made the scoring of cluster uncertain. This probably resulted in the fewer than expected number of cluster plants, but the deficit is not considered serious enough to alter the recombination estimate. The difficulty with cluster was most pronounced with yellow-green plants of strains 31A, 30B, and 30A in Table 1. These progenies were examined for the presence of double recombination phenotypes. Only one plant (from strain 30B) was classified as a double, being *ygyg Rr clcl*. Progeny testing, however, showed it to be a parental *ygyg RR clcl* genotype.

TABLE 3

F_2 recombination within Cl1-r1 *for the D_h/D_h linkage, coupling phase*
Cl1-r1/cl1-R1 *F_1 constitution**

Strain (Substrains)	Phenotypic segregation					Recombination among substrains percent
	r-Cl-* parent	r-clcl	RR Cl-	RR clcl parent	Recombination estimate percent	
30A F_2 (1,2,3)	434	31	50	95	14.7	14.1, 16.2, 17.3
28A F_2 (1,7)	255	25	32	59	16.0	15.5, 18.2
6–26B F_2 (5)	145	13	11	31	14.5	.....
Total	834	69	93	185	15.43	
Major Gene segregation		*Cl:cl*	P = 0.01–0.001†			
		r :R	P = 0.20			
		Linkage	P = 0.01–0.001			

* The incompletely dominant *R1* permits ready separation into the three phenotypes, rr, Rr, and RR. In order to make use of F_2 coupling efficiency, the Rr phenotypes were grouped with rr. This assumes that the linkage was coupling.
† Most of the deficiency can be attributed to Strain 30A since some of its yellow-green parental type progeny were not scored for cluster expression.

The D_h/D_h linkages scored in these diverse intra-*hirsutum* strains show 14–18 percent recombination between *Cl1-r1*, 14–20 percent for *r1-Yg1*, there being no double recombinants. Whether the double recombination was actually zero, F_2 being inefficient for detecting doubles, remains for additional three-point backcross testing to probe. Nevertheless double recombination was much less than the 2.0–3.6 percent theoretically possible if no interference were assumed, there being no doubles in a total of 1500 plants.

This evidence conformed to STEPHENS' (1955) suggestion that double recombination within a chromosome arm of *G. hirsutum*, on the average, is infrequent.

Recombination within the Cl1-r1-Yg1 *region in transferred diploid linkages*

The diploid linkages obtained from diploid D_5 and D_1 are very similar to the D_h/D_h linkage used in Section 1. The anthocyanin alleles of both diploids may be considered to be similar to *r1 in* phenotype, while the *Cl1* and *Yg1* alleles are equivalent to the D_h dominants. The D_5 linkage *Cl1-r1^{Rai} -Yg1-Dw*, was reported by RHYNE (1957) and the D_1 linkage was suggested by RHYNE (1955, 1957). The *r1* allele from D_1 has been established by GERSTEL (1956) in studies of R1 segregation in synthetic hexaploid hybrids. The D_1 linkage of *Cl1-r1-Yg1* is proven below in the B subsection. Both diploid linkages used herein were transferred to the D_h genome in a number of genotypic backgrounds.

A. D_5/D_h recombination in the *Cl1-r1-Yg1* linkage group.

An eight backcross generation transfer having the D_5 linkage in a *G. hirsutum* genotype was crossed with the standard *cl1-R1-yg1-dw* tester. Two backcrosses were made to separate the common *Yg2* allele of the A_h genome from the *Yg1* of the D_5 linkage. In the tenth backcross strain 28A contained the D_5 linkage as (*r2-yg2/r2-yg2*) *Cl1-r1^{Rai}-Yg1-Dw/cl1-R1-yg1-dw*, the recombination as shown in Table 4 being 5.5 percent in the *cl1-r1* region. This value is smaller than the

TABLE 4

Recombination in the D_5 linkage when transferred to G. hirsutum *strains, coupling phase* Cl1-r1^{Ra1} Yg1/cl1-R1-yg1

Strain (Substrain)	Phenotypic segregation: r-Cl1- parent	r-clcl	RR Cl-	RR clcl parent	Recombination percent	Recombination among substrains percent
28A F_2 D_5 linkage (2,5,6)	288 (247)	6 (23)	9 (23)	60 (47)	5.5 (14.0+ if D_h P that D_5 = 14.0% = 0.01–0.001)	0.0, 5.2, 10.5
	r-Yg-	r-ygyg	RR Yg-	RR ygyg	Recombination	
28A F_2 D_5 (2,6)	234 (206)	10 (20)	6 (20)	51 (56)	5.7 (14.0+ if D_h P that D_5 = D_h is 0.01–0.001)	5.5–6.4
28A $5F_2$*	47	0	2	10	0.0	
28A BC D_5 (2,6)	45	2	0	49	2.1	2.0, 2.2
28A 5 BC*	30	0	20	6	0.0	

* 28A 5 had Yg2 and Yg1 of D_5 as *r2-Yg2/r2-yg2 Cl1-r1^{Ra1} Yg1/cl1-R1-yg1*, giving duplicate factor segregation.

14 percent value found in the D_h/D_h linkage for the intra*hirsutum* F_2, and less than the 16 percent value for the D_h/D_h linkage in the interspecific 28A F_2 (see Table 3).

The D_5/D_h recombination between *r1* and *Yg1* in strain 28A (2 and 6) was 5.7 percent as seen in Table 4. Backcross data indicates 2.1 percent recombination for a small population. Either of these recombination values are different from the 14 percent figure found for the *r1-Yg1* region in D_h/D_h intra*hirsutum* material.

In the absence of a D_h allele to *dw* there is no estimate of D_h/D_h recombination for the *Yg1-dw* region. The D_5/D_h value ranged from 20–30 percent for *Yg1-Dw* but its significance cannot be evaluated.

The D_5/D_h linkage group showed lower recombination than did the D_h/D_h linkage groups in similar genotypic backgrounds. The D_5/D_h recombination is considered to be 2–9 percent in the *cl1-r1* region and 2–5 percent for *r1-Yg1*. Corresponding values for D_h/D_h are 15.43 percent and 18.57 percent. The gene order is clearly the same as the D_h lingage, i.e., *Cl1-r1-Yg1*, there being no evidence of any inversion of gene order. There were no double recombinations.

B. D_1/D_h recombination in the *Cl1-r1-Yg1* linkage group.

The D_1 linkage *Cl1-r1-Yg1* was extracted in a third backcross on *G. hirsutum* from the synthetic amphidiploid $2(A_2D_1) \times$ *G. barbadense* $2(A_hD_h)$. The data reported in Table 5 represent a hybrid with the standard *cl1-R1-yg1* tester when backcrossed with an appropriate *Cl1-r1-yg1* tester. Strains 6–25B and 6–26B had *Yg2* from A_2 and *Yg1* from D_1, separation by the backcross procedure not being successful. Their genotype, *Yg2/yg2 Cl1-r1-Yg1/cl1-R1-yg1*, gave duplicate factor segregation for yellow-green in the expected 3 *Yg*:1 *yg2yg2yg1yg1* ratio. Only one recombination was observed, it being a *r1r1yg1yg1(yg2yg2)* genotype. The

TABLE 5

Recombination at r1-Yg1 *in the D_1 linkage when transferred to* G. hirsutum *strains, coupling phase* Cl1-r1-Yg1/cl1-R1-yg1 F_1, *backcross segregation**

Strain (Substrain)	Phenotypic segregation					Recombination among substrains percent
	rr-Yg- parent	rr-ygyg	Rr Yg-	Rr ygyg parent	Recombination percent	
	Yg1 of D_1 linkage segregating					
6–26B BC (3 and 6)	64	0	1	51	ca 2.0	0.0, 2.5
	(56.8)	(1.2)	(1.2)	(56.8)	(If 2.0)	
	If 2.0%=D_1 value, P=0.30–0.50					
	Yg1 of D_1 linkage and Yg2 of A_2 linkage segregating=duplicate factor ratio					
6–26B BC (2 and 4)	48	0	31	18	ca 2.0	0.0, 0.0
	(48)	(0.5)	(24.7)	(23.8)	(If 2.0)	
6–25B BC (1,2,3,6)	293	1	158	129	ca 2.0	0.0, 0.0, 2.0, 0.0
	(287.6)	(2.9)	(148.2)	(142.3)	(If 2.0)	

* Backcross parent was *r2-yg2 r1-yg1 G. hirsutum* tester, (no *cl1* available).

recombination in the *r1-Yg1* region of the D_1/D_h linkage was much smaller than the 14 percent value observed for the D_h/D_h linkage (Table 1). The recombination is probably less than two percent as reported in Table 5.

The separation of *Yg2* from *Yg1* of D_1 was accomplished in strain 6–26B (3 and 6), which had a genotype *(yg2/yg2)Cl1-r1-Yg1/cl1-R1-yg1*. The backcross data is reported in Table 5, where a single recombination genotype, *R1-Yg1/r1-yg1*, is listed for the D_1/D_h linkage. Recombination presumably is less than the two percent value reported in Table 5 for the D_1/D_h linkage and clearly not 14 percent or greater as in the D_h/D_h linkage (Tables 1 and 2).

The recombination between *Cl1-r1* for D_1/D_h linkage was not obtained in the 6–26B and 6–25B families because of the absence of a suitable *cl1-r1* tester at Iguala for backcross testing. Their F_2 were grown but the presence of duplicates *Cl2* (of A_2) and *Cl1* resulted in a 15 *Cl* to 1 *clcl* duplicate factor ratio. The estimate of *Cl1-r1* recombination for the D_1/D_h linkage is, therefore, obtained from a 1955 backcross study.

The D_1 linkage *Cl1-r1-Yg1* was studied in a second backcross of *G. hirsutum* × amphidiploid 2(A_2D_1). The heterozygote *Cl1-r1-Yg1/ cl1-R1-yg1* when backcrossed with a *Yg2 cl1-r1-yg1* tester supplied the data summarized in Table 6. The 6.7 percent recombination for *Cl1-r1* in the D_1/D_h linkage is clearly less than that reported for the D_h/D_h linkage (Table 3) in both intra*hirsutum* and interspecific background, and less than 15–18 percent obtained in similar D_h/D_h studies made in 1955. The recombination in the D_1/D_h linkages is considered to be 2–9 percent for *Cl1-r1* and 0–2 percent between *r1-Yg1*. These values perhaps represent overestimates in view of the complication with *Yg2* and *Cl2* (ofA_2) in the backcross segregation.

A_h/A_h recombination for the R2-Yg2-Lc1 *linkage group*

The A_h linkage has been reported by STEPHENS (1955) as *R2-Yg2-Lc1-N*, *Cl2* being linked with *R2* but not placed relative to *Yg2*. He indicated *R2-Yg2* recom-

TABLE 6

Recombination at r1-Cl1 *of the D_1 linkage when transferred to* G. hirsutum *strains, coupling phase* Cl1-r1-Yg1/cl1-R1-yg1 F_1, *backcross segregation**

Strain (Substrain)	Phenotypic segregation: rr Clcl parent	rr clcl	Rr Clcl	Rr clcl parent	Not scored†	Recombination percent	Recombination among substrains percent
5–26A BC	270	13	27	286	92	6.7	8.9, 1.9, 4.7, 6.6
(1,2,3,4)	(278)	(20)	(20)	(278)		(If 6.7)	
	$P = 0.10$ that 6.7% is the D_1 recombination value						
	$P = 0.001$ that 14.0% is the D_1 value						
Major Gene segregation	*r:RR*	$P = 0.20$					
	Cl:clcl	$P = 0.90$					
	linkage	$P = 0.01$–0.001					

* A *r2-Yg2 r1 r1-yg1* stock of *G. hirsutum* served as backcross parent.
† Unscored plants were present as a portion of the population has been discarded because of burn from over-application of NH_4NO_2 fertilizer.

TABLE 7

Recombination among backcross progenies of the A_h linkage in G. hirsutum *strains having segregating major genes of* G. barbadense, *coupling phase* R2-Yg2-Lc2-n/r2-yg2-lc1-n F_1*

Strain (Substrain)	Phenotype segregation: Rr Ygyg parent	Rr ygyg	rr Ygyg	rr ygyg parent	Unscored plants†: Yg	ygyg	Recombination percent	Recombination among substrains percent
6–23B BC	90	14	29	35	3	59	25.6	21.7, 26.8, 28.4, 28.4
(1,4,6)	(95.2)	(9.8)	(23.8)	(29.2)			If 20.0=A_h	
	Ygyg Lclc parent	Ygyg lclc	ygyg Lclc	ygyg lclc parent				
6–23B BC	73	46	12	35	3	61	34.9	37.1, 41.7, 26.8
(1,4,6)	(80.9)	(38.1)	(15.0)	(32.0)			If 32.0=A_h	
6–23B 3 BC	7	18	17	9	..	..	31.3‡	

* Plants actually were random F_2 backcrossed to *r2-yg2-lc1-n* tester of *G. hirsutum*. Six double recombinations were observed.
† Early infestation by boll weevil prevented the scoring of the flower character in late flowering plants. Yellow-green plants are usually later than *Yg* (normal).
‡ Repulsion F_1 *R_1-Y2-lc1/ R_2-yg2-Lc1* genotype, itself a double recombination.

bination was 20–22 percent and *Yg2-Lc1* was 28 percent (although 32 percent was obtained in his backcrosses). This A_h linkage was transferred from a common tester to a genotypic background having both *G. barbadense* and *G. hirsutum* genes. This was done in order to evaluate any influence of background genotype on recombination in the A_h linkage, and also to accumulate by using F_2 plants any genes having a specific effect on chromosome pairing. (MENZEL and BROWN (1955) report the segregation of such genes in the F_2 of *G. hirsutum* × *G. barbadense*). This F_2 was of the genotype *R2-Yg2-Lc1-n/ r2-yg2-lc1-n*, *n* of A_h being substituted for *N* in the elimination of *Yg1* from D_h. Table 7 records the recombination data within *R2-Yg2* when these randomly chosen F_2 plants were pollinated with recessive *r2-yg2-lc1-n*. The value obtained, 25.6 percent for this interspecific background, is not different from the 20 percent value reported by STEPHENS (1955) for the same A_h/A_h linkage within *G. hirsutum* background.

The recombination at *Yg2-Lc1* for the same backcross is also given in Table 7. The first item, second grouping, represents the coupling phase which gave a 34.9 percent recombination value for this A_h/A_h linkage. The second item is the repulsion phase data showing 31.3 percent recombination. Either value is in close agreement with the 32 percent value reported by STEPHENS (1955) within a *G. hirsutum* genotype.

Six double recombinations were observed, two of which were *R2-yg2-Lc1* types. Doubles were more frequent than reported by STEPHENS (1955). The influence, if any, of the interspecific genotype enhanced rather than decreased double recombination. No evidence of asynaptic plants having reduced chiasmata frequencies was observed. The A_h/A_h linkage is considered to have 20–26 percent recombination at *R2-Yg2*, 28–35 percent at *Yg2-Lc1*, doubles resulting despite interference.

A_2/A_h recombination in the R2-Yg2 *linkage group*

The diploid linkage of *G. arboreum* (A_2) is composed of *Cl2-R2-Yg2-lc1 (+n)*, of which *Cl2*, *lc1*, and *n* need further study. The *R2-Yg2* recombination is here reported for third and fifth backcross F_3 segregation, although first, second, third and other backcross generations have given similar results. RHYNE (1955) showed a nine percent recombination within *R2-Yg2* in a tetraploid interspecific F_1, A_2D_1/A_hD_h, this departure from the 20 percent value reported by STEPHENS (1955) for the A_h linkage being typical of all backcross generations.

Several different strains were used for the data in Table 8, each having the essential test genotype *R2-Yg2* of $A_2/r2\text{-}yg2$ of A_h in interspecific backgrounds. As mentioned above, the purpose in using F_2 plants was to accumulate any specific genes influencing chromosome pairing. None of the progenies, however, showed

TABLE 8

Recombination in the A_2 linkage at R2-Yg2 *in F_3 progenies when transferred to* G. hirsutum *strains, coupling phase* R2-Yg2/r2-yg2*

Strain (Substrain)	Phenotypic segregation					Recombination among substrains percent
	R-Yg- parent	R-ygyg	rr Yg-	rr ygyg parent	Recombination percent	
6–21A3 3BC F_3	55	1	3	20	6.4	
6–22A 3BC F_3 (6,7,1)	207	5	16	82	5.7	5.7, 4.2, 12.1
6–24A6 3BC F_3	108	2	5	34	5.4	
6–22B3 5BC F_3	233	19	18	61	12.1	
Total	603	27	42	197	7.9	
	(574)	(78)	(78)	(139)	If 20.0%=A_2 recombination	
	P = 0.001 that 7.9 of A_2 = 20.0% of A_h					
	Yg1 and Yg2 present, Duplicate Factor 15:1 ratio expected					
6–24A 5 3 BC F_3 (5,2,4)	415	3	134	43	ca 6.0	0.0, 0.0, 13.1

* The *R2* dominant red plant parts is similar to *R1* in expression. The *R2* came from two distinct types of *G. arboreum*, the 24A transfer is readily separable from the other transferred type.

any evidence of asynapsis. All strains showed an A_2/A_h recombination of 7.9 percent for the *R2-Yg2* region much reduced from the 20 percent value of STEPHENS in pure *G. hirsutum* or the 25.6 percent figure cited above for the A_h/A_h linkage in an interspecific genotype.

Several strains had the test genotype *R2-lc1/ r2-Lc1* from which backcross and F_2 progenies were grown. The recombination within this region for each strain was not different from STEPHENS' (1955) value of 52 percent in the A_h linkage and the 50+ percent reported above; the reduction in recombination to 37–45 percent for the cumulative population is significant. Thus, the reduction in recombination for the entire region *R_2-Yg2-lc1* can be assumed to be that reduction demonstrated for just the *R_2-Yg2* sector. Further studies are needed to indicate whether there is actually less reduction in recombination per unit length in the *Yg2-lc1* region than in the *R_2-Yg2* region of the *R_2-Yg2-lc1* linkage.

DISCUSSION

In order to evaluate genotypic influence on genetic recombination a *G. hirsutum* linkage group was transferred to a number of inter- and intraspecific backgrounds. There was no evidence for any strain influence on recombination, e.g., the D_h *Cl1-r1* recombination was 14–18 percent in the intra*hirsutum* strains, 14.5 percent in an interspecific strain having both A_2 and D_1 genes, and 15–18 percent in an interspecific strain having A_h, D_h and D_5 genes (see Table 3); each strain could be considered statistically a part of the larger population having 15.43 percent recombination. Similarly the several strains of *G. hirsutum* showed recombination ranging from 14.2 to 28.5 percent within the D_h linkage *r1-Yg1*, each strain value (Table 1) being considered a part of a larger *G. hirsutum* population having 18.57 percent recombination. Likewise, the diverse interspecific strains having genes from A_h, A_b, D_h, and D_b showed 20–26 percent recombination within *R2-Yg2* and 26–41.7 percent between *Yg2-Lc1* of the A_h linkage, but these values were not different from the intra*hirsutum* recombination values reported by STEPHENS (1955).

On the other hand LEVINE and LEVINE (1955) report genotypic influence by strains on total crossing over percentages as well as recombination within particular regions of the third chromosome of *Drosophila pseudoobscura*. Their significant differences amounted to 2–3 percent in tight linkages and 2–7 percent in loose linkages. This magnitude of genotypic influence perhaps could be shown for the Gossypium strains used in this study, provided larger populations were available. The range in recombination, 4.2–12.1 percent, within *R2-Yg2* among strains having the A_2/A_h linkage (Table 8) presumably reflects some strain influence on recombination, as does the range, 21.7–28.4 percent, for the A_h/A_h linkage (Table 7). The population sizes, however, were inadequate for detecting any differences between strains.

According to BROWN and MENZEL (1954) and MENZEL and BROWN (1955) specific genes affect metaphase chiasmata frequencies of some interspecific Gossypium hybrids, the action of these genes resulting in reduced recombination

in the hybrids. Evidence for such genes was not observed in any of the progenies analyzed in the course of this research. The uniformity of recombination within and between strains indicate the absence of such genes, since segregation among randomly chosen plants would have occurred had they been present (assuming, of course, that any reduction in metaphase chiasmata reflects a reduction in synapsis at pachytene rather than precocious terminalization of chiasmata at metaphase. If the genes were desynaptic in action no relationship to reduce crossing over might be expected). The high fertility of the plants used in the present study suggest that metaphase chromosome behavior was normal, as unequal chromosome segregation in cotton is often accompanied by infertility.

The transference of a linkage group from each of three diploid species to *G. hirsutum* results in a significant decrease in genetic recombination for factors within the linkage under study, (Tables 4, 5, 6 and 8). The change in number and/or position of chiasmata responsible for these observed results must be attributed to differential structural evolution of the chromosomes of the several species involved in the study. Cryptic chromosome structural differentiation was cited by GERSTEL (1956) to explain the allopolyploid type of segregation found in synthetic hexaploids *G. hirsutum* × *G. raimondii* $2(A_hD_hD_5)$ and *G. hirsutum* × *G. thurberi* $2(A_hD_hD_1)$. Two independent genetic procedures, therefore, show evidence of structural differentiation which cannot be detected cytologically. Both studies support STEPHENS' suggestion (1950) that speciation in Gossypium has proceeded by structural differentiation of the chromosomes.

The nature of the structural difference between chromosomes is assumed to be usually one of accumulation of small inversions and translocations. The cytological consequences of the differentiation is not easily described. As pointed out above the decrease in recombination observed in this study can be explained either by a reduction in total chiasmata or by a shift in position or location of the chiasmata along the length of the chromosome.

The average per bivalent chiasma frequency for *G. hirsutum* is given by MENZEL and BROWN (1955) as 1.80–1.95. In a D_5/D_h hybrid showing a 24–26 percent reduction in genetic recombination for the *Cl1-r1-Yg1* linkage, one might expect a cytologically detectable 12–13 percent reduction in chiasmata frequency. But average chiasmata counts for a variety of interspecific hybrids (including $A_hD_h \times D_5$) show no appreciable reduction in chiasmata frequency from that of *G. hirsutum*. On the contrary, the data of MENZEL (1955) (by the use of a translocation technique that enabled her to count chiasma at particular points along the chromosome) show that a decrease in chiasma at one point was accompanied by an increase at an adjacent position. Therefore the available cytological evidence does not support a change in chiasmata frequency hypothesis, but does support a change of chiasma position explanation as the mechanism altering recombination in this material.

A definitive genetic test of this question must await construction of hybrids in which an entire chromosome (or chromosome arm) is tagged with suitable markers, thus making possible a region by region evaluation of genetic recombi-

nation. The material used in the present study was not adequate for this purpose.

The evidence presented in this paper, indicating a reduction in genetic recombination between homologous linkages of interspecific hybrids, is of importance to plant breeders, especially to the cotton breeder who is looking toward related cotton species as sources of genetic variability. During interspecific transference from related species to cultivated cottons, genes will tend to be inherited in blocks rather than being individually separable by common breeding techniques. Whether such a block inheritance of chromosome segments will be an advantage or a hindrance to cotton breeders will depend, of course, on whether a given factor in which the breeder is interested is associated with desirable (or neutral) factors or deleterious genes.

The cotton breeder interested in exploiting genetic variability from related species needs information on the occurrence of desirable and undesirable blocks of genes and on techniques for obtaining the separation of the desirable from the undesirable genes. Studies such as those by GERSTEL (1956) and RHYNE (1951), showing differences in segregation (i.e., genetic recombination) in hybrids between *G. hirsutum* and related diploid species suggest that some species may be more desirable than others as sources of variability. Special techniques are therefore required for obtaining recombination within these blocks of genes that will be encountered in transferring characters from the diploids to cultivated types. Some of these might be: (1) The use of strain genotypes that are known to have relatively high genetic recombination frequencies; these, when used in conjunction with large populations, will enhance the recovery of desired recombinants. (2) The use of blocks of genes that shift chiasma from one portion of the chromosome to a segment in which recombination is desired. MENZEL (1955) showed such a cytologically detectable shift of chiasmata from one point to another when the D_5 chromosome was compared with its D_h homologue. (3) The use of both (1) and (2) in conjunction with irradiation techniques.

SUMMARY

Observed changes in genetic recombination in interspecific Gossypium hybrids have been attributed to both chromosomal differentiation and genotypic influence accruing in the evolution of the species. The present study was designed to evaluate the relative importance of chromosomal structural differentiation to genotypic influence on genetic recombination. In order to evaluate the influence of strain genotype two domestic linkages of *G. hirsutum* were transferred to a number of diverse strain genotypes, both inter- and intraspecific backgrounds being used. Genetic recombination was estimated by F_2 and backcross progeny techniques. The population sizes available did not yield statistical evidence for any genotypic influence on recombination, i.e., the strain to strain variation for a specific linkage group was representative of sampling variation from a large uniform population.

In order to evaluate the influence of chromosome structural difference, on recombination, three diploid linkage groups were transferred to one of the genomes of domestic *G. hirsutum* in a range of strain genotypes. Their recombination

in the transferred linkage groups was consistently less than that obtained for the domestic linkage in similar genotypic backgrounds. The sizable and significant reduction in genetic recombination conforms to STEPHENS' (1950) suggestion that speciation among Gossypium species has occurred both by cryptic structural differentiation of chromosomes as well as by major gene substitution and mutation. In the writer's opinion, these results should be interpreted to mean that chromosomal structural differences in the interspecific hybrids are more important in altering genetic recombination than is the influence of genotypic background. The nature of the chromosomal structural differentiation causing a reduction in recombination has been discussed as a change in chiasmata frequency and/or position.

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ACUTE RADIATION RESPONSE OF MICE FROM A CROSS BETWEEN RADIOSENSITIVE AND RADIORESISTANT STRAINS[1]

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THE existence of genetic differences in the radiosensitivity of inbred mouse strains has been well established under controlled experimental conditions (KOHN and KALLMAN 1956; GRAHN and HAMILTON 1957). These studies have indicated that strain BALB/c is the most radiosensitive of those investigated and that strain C57BL is relatively resistant. As a consequence of this known difference, strains BALB/c and C57BL were selected for a study designed to give information on the mode of inheritance of radiosensitivity.

In toxicity testing, observed sensitivity is assumed to be a continuously distributed variable (MATHER 1946). When rectified, this distribution is described by the dosage-mortality slope—the latter being inversely proportional to the standard deviation of the distribution. The mean or median of the distribution (LD_{50}) and a measure of dispersion (the dosage-mortality slope) will be used in this study to describe the acute lethal response. The slope has previously been employed in the estimation of phenotypic variances for inbred strains (GRAHN and HAMILTON 1957) and should provide, for segregating generations, a means of estimating the genetic variance.

MATERIALS AND METHODS

Both reciprocal F_1 generations were produced from parent strains BALB/c and C57BL/6. These are designated as the CB F_1, from the BALB/c dam, and the BC F_1, from the C57BL/6 dam. Second generation hybrids were produced from each reciprocal F_1 by mating *inter se* and are designated as the CB and BC F_2. A small group of F_3 mice was also produced. In this instance, the matings were made within the five coat color types that segregated in the F_2. One additional group of animals was produced from an outcross of the F_1 dam with the strain A/Jax sire ($F_1 \times A$). Though these mice were not originally intended to be a part of the present study, they provide some useful information.

The mice were exposed to single doses of whole-body 200 kvp X-irradiation when 60–110 days of age. A summary of the experimental design is given in Table 1. A series of independent tests to estimate the LD_{50} values was carried out on the several generations; 17 for the BALB/c, 16 for the C57BL/6, 20 for the F_1, 15 for the F_2 and 5 for the F_3. Each test generally consisted of three dose groups with an average of 14–15 mice per dose. The average within-test regression of arcsine-mortality on dosage in roentgens (dosage-mortality slope) was estimated

[1] This work was performed under the auspices of the U.S. Atomic Energy Commission.

TABLE 1

Summary of experimental design: First number, number of independent tests employing given dose; second number, total number of mice; third number, percent mortality from pooled test results

Dose	BALB/c	C57BL/6	F_1	F_2	F_3
420	4–59–7	.....	.....	.....	.....
435	4–60–15	.....	.....	.....	.....
450	4–60–25	.....	.....	.....	.....
(465)	.....	.....	.....	.....	.....
480	4–53–38	.....	.....	5–75–4	.....
495	4–58–41	.....	.....	.....	5–72–13
510	4–50–52	.....	.....	6–85–2	.....
525	4–57–61	.....	.....	.....	.....
540	4–51–59	.....	.....	6–89–16	.....
555	4–54–89	.....	8–113–1	.....	.....
570	4–57–84	6–89–10	.....	7–95–27	.....
585	4–54–87	6–87–23	9–131–17	.....	.....
600	4–59–95	6–89–20	.....	.....	5–71–46
615	.....	6–82–32	9–131–20	.....	.....
630	.....	6–81–56	.....	7–103–55	.....
645	.....	6–84–57	9–128–41	.....	.....
660	.....	6–88–74	.....	.....	.....
675	.....	6–87–89	9–130–56	.....	.....
690	.....	.....	.....	6–83–71	.....
705	.....	.....	7–106–69	.....	5–72–82
720	.....	.....	.....	6–86–83	.....
735	.....	.....	6–88–84	5–75–92	.....
750	.....	.....	5–69–96	.....	.....
Total mice	672	687	896	691	215

by a weighted covariance analysis for each group. This slope is used to derive the LD_{50} value for each test.

The earlier investigation of the parent inbreds (GRAHN and HAMILTON 1957) had indicated that age was an important variable in the acute lethal response while the litter seriation and litter size associated with the exposed mice were of little consequence. On the basis of those results, only the relationship of age to mortality was derived for the F_1 and F_2 generations. The F_3 data were not sufficient to warrant an estimate.

The regression of mortality on age can be used to predict expected mortality at any age within the limits of the data (60 ~ 110 days). If we assume the dosage-mortality slope value to be constant in the above age range, then $LD_{50/30}$ values can be estimated from the predicted mortalities. Age changes in sensitivity are given below (see Figure 1) in terms of the LD_{50} value because of its clarity of expression.

The linear regression of mortality on age was used to adjust test responses to a constant age at exposure of 85 days. The individual test LD_{50} values were then estimated from the age-adjusted test means. The standard error of the LD_{50} was

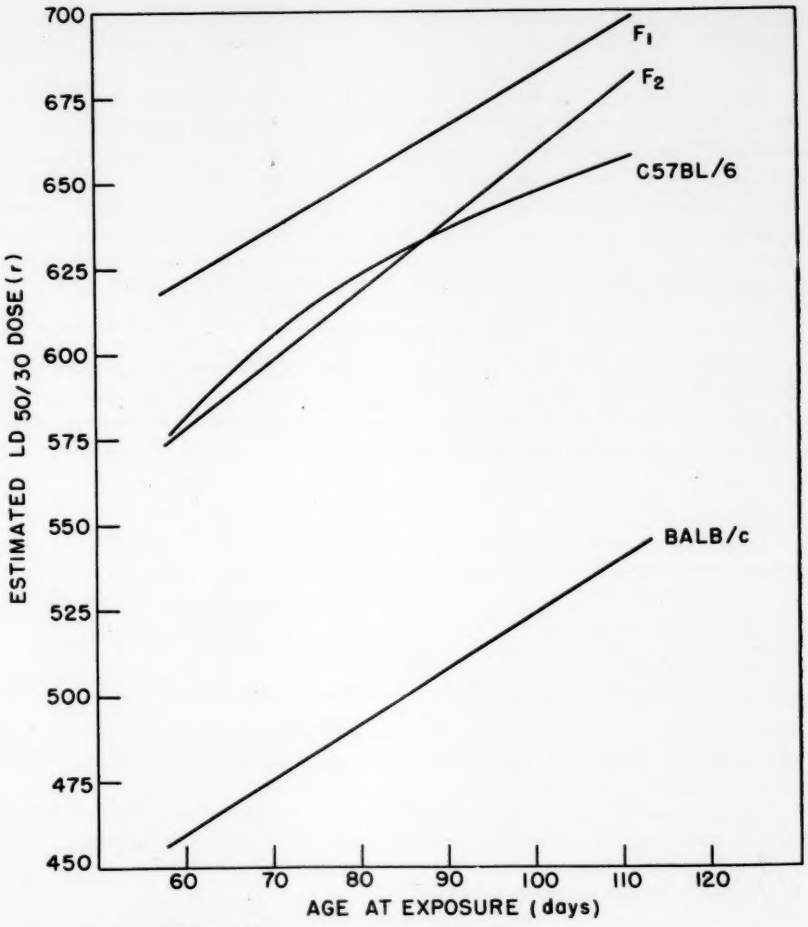

FIGURE 1.—Dependence of $LD_{50/30}$ value on age at exposure.

derived from the distribution of the test estimates. A more complete description of the statistical procedures and methods of distributing the mice among the doses in each test was given in the previous report (GRAHN and HAMILTON 1957).

In order to estimate the LD_{50} values for certain subclasses, i.e., sex, reciprocal crosses, and coat color type, the data for the subclass in question were pooled within dose across all individual tests. Estimates of the dosage-mortality slope, the LD_{50} value and their standard errors were derived from these pooled data by a weighted least squares linear regression analysis. The method of pooling was

required since the within-test within-dose subclass sample sizes were too small (generally fewer than eight mice per test dose) to permit the form of analysis described above for the principal estimates.

With reference to the mortality data given in Table 1, it may be noted that these are also pooled data figures. Because the data are nearly equally distributed on either side of the 50 percent mortality point, LD_{50} doses derived from these data will vary by only a few roentgens from the mean test values given in this study. However, slope values obtained from pooled data are consistently lower than the within-test estimates, since pooled data estimates are not independent of variation among tests. Nevertheless, the comparative magnitude of the slopes in the different generations and their linearity can be readily checked.

First generation hybrids

The $LD_{50/30}$ dose at 85 days of age is estimated to be 659.5±6.6r and the within-test dosage-mortality slope is + 0.404±.028. (These values are presented in Table 4 with the parental values for comparative purposes.) A breakdown of the F_1 mice into sex and reciprocal subclasses is given in Table 2. A significant sex

TABLE 2

$LD_{50/30}$ values by sex and reciprocal for BALB/c : C57BL/6 F_1 hybrids

Reciprocal cross	Female	Male	Combined sexes
CB (BALB/c ♀ × C57BL/6 ♂) F_1	676.4r	648.3r	661.6r
BC (C57BL/6 ♀ × BALB/c ♂) F_1	675.8r	654.1r	662.4r
Combined reciprocals	676.7±7.0r	651.5±5.3r	

difference ($P < .025$) is apparent; the female has an LD_{50} 25.2±8.8r higher than the male. No significant sex differences were detected in the parent strains (GRAHN and HAMILTON 1957).

There is no difference between reciprocals, within or across sex, which indicates that no maternal influence or sex linkage can be detected in the response.

The LD_{50} dose increases linearly with age between 60 and 110 days; rising from 622r to 696r in that interval (Figure 1).

Second generation hybrids

The F_2 LD_{50} value is 627.8±6.6r. The within-test dosage-mortality slope is + 0.274±.026. The sharp drop in the slope value (see Table 4) is an indication of increased variability, most of which can be assumed due to the genetic variation expected in a segregating generation. There are no sex or reciprocal differences in the F_2 mice. Age changes in the LD_{50} in this generation also appear linear with age and are given in Figure 1.

The F_1 mice are heterozygous for agouti, black and albino (*AaBbCc*). The F_2 generation segregates five coat color types: black agouti, brown agouti, black, brown and albino in an expected ratio of 27:9:9:3:16, respectively. Nonagouti,

black and full color are derived from the C57BL parent, and agouti, brown and albino come from the BALB/c parent.

To examine the possibility of a non random segregation or "association" of sensitivity factors with the color genes, the F_2 mice were subdivided into the color classes and comparisons were made on an alternative allele basis with reference to the parent inbred that contributed the gene. The results are given in Table 3. Only minor differences are apparent at the agouti and black loci, but a

TABLE 3

$LD_{50/30}$ values for coat color comparisons in BALB/c : C57BL/6 F_2 number of mice of given color type shown in parentheses

BALB/c contribution	C57BL/6 contribution	Difference
Agouti (384)	Nonagouti (134)	
640.1r	635.5r	4.6r
Brown (157)	Black (361)	
635.6r	642.0r	6.4r
Albino (173)	Nonalbino (518)	
602.3±8.6r	639.3±7.9r	37.0r*

* Difference signicant at one percent level.

TABLE 4

Comparative data of parental and hybrid generations

Strain	Observed $LD_{50/30}$ ± SE	Observed slope ± SE	Reciprocal slope
BALB/c	500.1±6.9r	.368±.032	2.72r
C57BL/6	630.3±4.1r	.516±.041	1.94r
F_1	659.5±6.6r	.404±.028	2.48r
F_2	627.8±6.6r	.274±.026	3.65r
F_3	613.3±28.0r	.264±.020	3.79r

significant difference is evident at the albino locus, with albino associated with sensitivity.

The relative sensitivity of the albino is confirmed in part by the results of the irradiation of the $F_1 \times A$ outcross progeny. Since strain A is homozygous recessive for the color genes involved (*aabbcc*), the progeny of this cross segregated 1:1 for albino and nonalbino. The LD_{50} for the albinos is 615r (116 mice tested) and for the nonalbinos it is 650r (119 tested). Because of the small samples involved, the difference is within the limits of one standard error. However, the magnitude and direction of the difference is similar to that seen in the F_2 segregants.

Third generation hybrids

The F_3 mice were produced principally to ascertain whether the F_2 mice were fully expressing their expected genetic variability as measured by the dosage-mortality slope. It was considered possible that the uniform vigor of the F_1 dam

could have suppressed the full expression of variation in the F_2. Additionally, the matings were made within the five color classes in an effort to obtain additional information on the albino effect on sensitivity.

The LD_{50} is 613.3±28.0r and the dosage-mortality slope is + 0.264±.020. The latter is nearly identical to the F_2 slope and indicates that the expression of genetic variability in the F_2 is not affected by maternal factors.

The F_3 mice included 56 albinos and 159 nonalbinos. These were distributed equally across the three test doses and gave mortalities of 46.4 percent and 47.2 percent for albino and nonalbino, respectively. Among the several color families, black agouti moved to a position of relative resistance (36 percent mortality) and brown agouti to a position of relative sensitivity (53 percent mortality). Thus, the F_3 data appear to bear little resemblance to those of the F_2. However, an adequate genetic analysis of this generation would require a very broad sampling of F_2 genotypes for matings within all color segregants. As this type of toxicity testing is relatively uneconomical for mice, the production of the F_3 was discontinued.

Comparative analysis of parent and hybrid generations

In Table 4, the observed LD_{50} values and dosage-mortality slopes (see also Figure 2) are presented for both parent strains and the three hybrid generations.

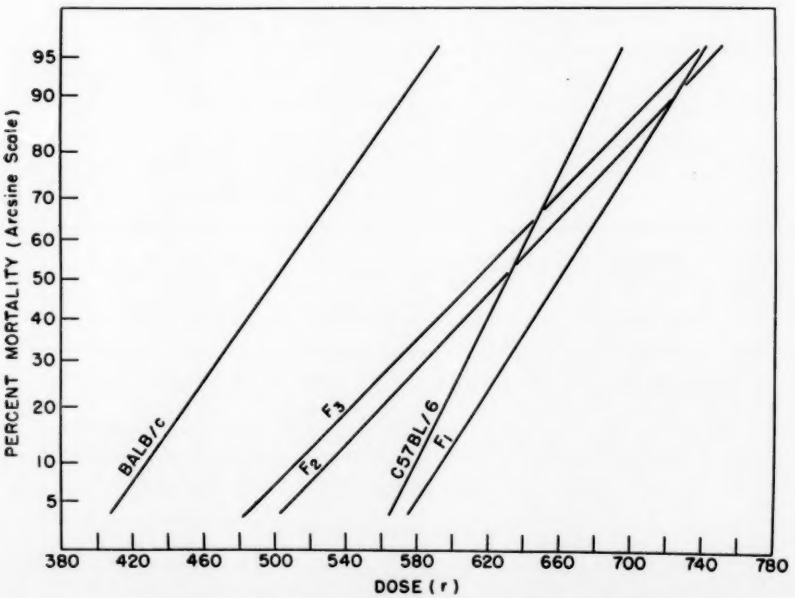

FIGURE 2.—Relationship of dosage-mortality slopes for parent and hybrid generations.

A comparison of LD_{50} values shows that the F_1, with an LD_{50} nearly 30r above the high parent, expresses a significant degree of hybrid vigor. In the F_2, the value is almost equal to the high parent, while in the F_3, the LD_{50} is slightly, but not significantly, below the F_2. These estimates suggest that, genetically, radioresistance is dominant to sensitivity, or, in other terms, is the function of a nonadditive genetic system.

The dosage-mortality slopes support a different genetic interpretation. In the F_1, the slope is intermediate to the two parents, while the F_2 and F_3 slopes fall below the low parent, and manifest a sharp increase in the variability of response. Since the squared inverse slope is directly proportional to the variance of the population, the slope data are typical of a characteristic controlled by an additive genetic system, wherein the F_1 variance is equal to the parental variance and the F_2 expresses the added variance due to genetic segregation.

Estimation of heritability of radioresistance

Heritability can be estimated from these data by the relation: $h^2 = (\sigma^2_{F_2} - \sigma^2_{F_1})/\sigma^2_{F_2}$. This assumes the F_1 variance to be an unbiased estimate of the environmental variance (σ^2_E), and the F_2 variance to include only additive genetic variance (σ^2_G) in addition to σ^2_E. The arithmetic mean of the parental variances also estimates σ^2_E.

The variances, in this instance, are derived from the dosage-mortality slopes. The arcsine slope can be converted to probits by taking the product of the arcsine slope and the regression of probit values on arcsine values (+ .0481) as described by GRAHN and HAMILTON (1957). The reciprocal of the squared probit slope provides a direct estimate of the variance of the distribution. The variances are: BALB/c, 3192; C57BL/6, 1624; F_1, 2652; F_2, 5761.

Using $\sigma^2_{F_1} = \sigma^2_E$, h^2 is 0.54. If σ^2_E is derived from the mean parental variance, $h^2 = 0.58$. These figures clearly indicate that a great amount of genetic variability in radiation resistance does exist in a heterogeneous population.

DISCUSSION

Resistance to the acute lethal effects of whole-body X-irradiation is a heritable characteristic that shows both additive and nonadditive genetic qualities. In terms of the LD_{50} dose, genetically determined resistance is nonadditive. This property was also present in the X-ray data of KOHN and KALLMAN (1956) for strains BALB/c, A/He and their F_1 hybrid. Dominance of resistance is, in fact, a general finding in the first generation offspring of crosses between resistant and susceptible strains within a number of plant and animal species when subject to test by a range of infectious or toxic agents (see review by GOWEN 1948). It should be emphasized, however, that in the F_2 of the present study, the degree of dominance varies with dose (see Figure 2). For example, an LD_{10} is 50r below the high parent, while an LD_{90} is 40r above it. A very incomplete picture is derived by using a single level of either response or dose, and genetic interpretations will vary accordingly.

It is unfortunate that the dose-response slope has not received more attention, since it can convey information beyond that noted above. In an F_2, a linear slope that is shallower than the F_1 or parent values provides a measure of genetic variability and also suggests that the character being investigated is under additive genetic control. This is seen in the present data and in the response of Drosophila to DDT (KING 1955). A nonlinear, or stepped, slope, such as observed by LICHTWARDT (1956) for the response of houseflies to DDT, indicates the presence of one or two major dominant genes.

In addition to the slope data, a second parameter, recovery rate, adheres to the additive scheme. For strains BALB/c, A/He and their F_1, KOHN and KALLMAN (1957) gave the following recovery rates as measured by the paired dose technique: BALB/c, 21 percent/day; A/He, 47 percent/day; F_1, 35 percent/day (percent recovery from a first dose of 350r).

To the extent of the limited available data, the additive component of resistance appears to find more general expression, while the nonadditive component seems limited to expression on the dose scale. This may indicate the latter component to be primarily a heterotic manifestation that acts to raise the dose-response threshold. A fully adequate interpretation of the nonadditive component cannot be made without data on the response of a wide variety of interstrain crosses.

The high heritability estimate of .54–.58 indicates that a program of selection for radioresistance or sensitivity could be successfully carried out, although the nonadditive contribution might obscure the results in early generations. Though no selection experiment has been attempted, SCHOTT (1932) reported successful selection for resistance to mouse typhoid, which has been shown to have a high genetic correlation with radiation resistance (GRAHN 1954; STADLER and GOWEN 1957). SCHOTT raised resistance from 18 percent to 75 percent in only six generations. This could only have been accomplished if heritability was high.

The significant association between the albino locus and radiosensitivity that was observed in the F_2 requires further evaluation. Its possible presence in the testcross mice ($F_1 \times A$) tends to implicate the albino gene itself, while the apparent loss of this association in the F_3 indirectly infers that a linkage could be involved. Because of the small sample sizes employed, and in the absence of a direct test for linkage, no positive genetic interpretation can be brought forth.

In view of the existing data, a program of recurrent backcrossing to the sensitive parent, BALB/c, has been instituted. This procedure should provide additional information on both the additive and nonadditive genetic components, and, as well, should help elucidate the relationship between albinism and sensitivity.

SUMMARY

1. The acute lethal response to whole body X-irradiation of F_1, F_2 and F_3 generations from parent strains BALB/c and C57BL/6 is shown to manifest both nonadditive and additive genetic effects. The variance of sensitivity, measured by the dosage-mortality slope, is additive. The $LD_{50/30}$ dose is nonadditive. It is

suggested that the nonadditive component may be primarily a heterotic expression.

2. No maternal influence or sex linkage is detected in the acute response.

3. Radiosensitivity is associated with the albino gene, although the data do not permit discrimination between pleiotropism or linkage as the basis of this association.

4. In the F_2, the genetic component of variance (heritability) accounts for about 55 percent of the variation in radiation sensitivity.

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RELEASE OF GENETIC VARIABILITY THROUGH RECOMBINATION. I. DROSOPHILA PSEUDOOBSCURA

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MENDEL discovered that genes segregate, rather than blend during the process of germ cell formation in heterozygotes. This makes sexual and asexual reproduction profoundly different in their genetic consequences. Asexual reproduction yields a progeny which, barring mutation, is genotypically uniform and identical with the mother. Sexual reproduction engenders genetic variability in the offspring. A variety of genotypes appear in the progeny of heterozygous parents, and these genotypes become available for the sorting-out action of natural or artificial selection. Herein lies the biological function of sexual reproduction; it creates a great variety of genotypes, perhaps so great that no genotype is likely to become embodied in more than a single individual. Under asexual reproduction genotypic diversity can arise only through occurrence of different mutations in different lines of descent. Compared to sexuality, this is a most inefficient method of producing genetic diversity.

All of the above is a matter of elementary genetic theory. But it is very little known just how much genetic variability can be released by recombination of genes available in a small sample of a sexual population. This problem bears directly on the disputed ground of the classical and balance theories of population structure (DOBZHANSKY 1955, 1957). If the classical theory is correct, then most individuals in sexual populations are homozygous for most genes, and a small group of individuals will carry only a few unfixed loci. On the other hand, under the balance theory, every individual is expected to be highly heterozygous; a small population sample will, then, contain many unfixed loci, and their recombination may yield considerable genetic diversity. According to the classical theory, the occurrence of an evolutionary change will usually require new mutations; according to the balance theory the recombination of the unfixed loci already available in a sexual population will be important and perhaps will eclipse the contribution of the new mutants.

This is the first of a series of articles describing experiments designed to produce information concerning the release of genetic variability through recombination. For this purpose we have chosen three species of Drosophila—*D. pseudoobscura*, *D. persimilis* and *D. prosaltans*, and have studied the recombination in their "second" chromosomes.

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Background of the present investigation

DOBZHANSKY (1946) examined the recombination products derived from three second chromosomes of *D. pseudoobscura* from Mount San Jacinto in California. The homozygotes for two of these chromosomes (B and C) were normal in viability at the three temperatures (16½°, 21° and 25½° C) at which they were studied, but the homozygotes from the third (A) were lethal at 25½°, semilethal at 21°, and almost normally viable at 16½°. Nevertheless, among the chromosomes obtained by crossing over between the three parental lines, some were semilethal or even lethal when homozygous. This was true even for some crossovers between chromosomes B and C. Some of the crossovers between A and B were lethal at 16½°, at which temperature neither A nor B were lethals, while at 25½° most of the recombination products acted as lethals when homozygous. Recombination of genes in two chromosomes neither of which is lethal may, thus, yield a chromosome lethal when homozygous. Such lethal recombination products were called *synthetic lethals*.

WALLACE *et al.* (1953) studied the recombination products between ten chromosomes from two of their experimental populations of *Drosophila melanogaster*. None of the ten were lethal when homozygous. They tested 4830 chromosomes which were recombination products between the ten original ones. Of these, 143, or 2.96 percent, were lethal when homozygous. Apart from the synthetic lethals, homozygotes of every degree of viability, from extreme semilethal to normal, were found among the recombination chromosomes tested. Furthermore, a quite considerable variability was discovered among the chromosome combinations tested—some of them did and others did not produce synthetic lethals and semilethals, some yielded recombination products of uniform and others of variable viability.

HILDRETH (1955, 1956) failed to obtain synthetic lethals. He tested 5186 possible recombination X chromosomes from intercrosses of ten different laboratory stocks of *Drosophila melanogaster*. The number of lethals observed was not significantly greater than among the chromosomes from the stocks themselves. This is not a very remarkable result since one third of the X chromosomes in a population are carried in males which would die if they had a sex-linked lethal. Therefore, X chromosomes capable of giving sex-linked lethals by recombination with other X's in the same population would be discriminated against by natural selection. This is not necessarily the case with autosomes, since the lethals produced by recombination would be carried mostly in heterozygotes, and might be removed by another recombination. Only synthetic lethals that would kill homozygous females, but not the males, are expected in the X chromosomes. An indication that such lethals may exist in Drosophila was obtained by KERR and KERR (1952), and lethals which kill homozygous diploid females but not haploid males are known in the honeybee (MACKENSEN 1951; LAIDLAW, GOMES and KERR 1956). It is not so easy to account, however, for HILDRETH's not finding synthetic lethals among 2771 crossover products of third chromosomes of *Drosophila melanogaster*. Although WALLACE *et al.* (1953) worked with the second chromo-

somes of the same species, it does not seem very likely that the two large autosomes, the second and the third, differ so sharply in their ability to yield synthetic lethals by recombination.

Even before HILDRETH's papers were published, PROF. E. SPIESS of the University of Pittsburgh, PROF. C. PAVAN and his collaborators at the University of São Paulo, and the authors of the present article decided to study the genetic variability induced by recombination in *Drosophila persimilis*, *D. willistoni*, *D. pseudoobscura*, and *D. prosaltans*. The experiments on *D. willistoni* have, unfortunately, broken down owing to a series of accidents, but those with the other three species have been carried through in accordance with a common plan. The results showed that synthetic lethals occur in every species tested, albeit with different frequencies. However, synthetic lethals are merely the extreme negative variants in the array of viabilities obtained as a result of recombination of genes carried in nonlethal chromosomes in natural populations. Our problem is broader than finding out the frequencies of synthetic lethals. The really basic issue is how much genetic variability affecting fitness, both favorably and unfavorably, is stored in natural populations and can be released by recombination.

Plan of the experiments

The participants in the projected study agreed beforehand to make the experiments follow a common plan. In each species, population samples were taken in two different localities, remote either in distance, or in ecological conditions, or in both. The chromosomes in these samples, one chromosome per wild fly, were studied for their effects in homozygous condition. This was done by means of a series of crosses designed to produce phenotypically distinguishable flies homozygous and heterozygous for the same chromosome derived from a natural population. The details of this technique will be specified below, separately for each species. In each species and population, it was found, as expected, that some of the "wild" chromosomes are lethal, or semilethal, or subvital, or normal, or, rarely, supervital in double dose. Among these chromosomes, a group of ten was selected from each population. The chromosomes picked out were mildly subvital to normal in homozygous condition; lethal, semilethal, sharply subvital and supervital chromosomes were deliberately avoided (although one of the chromosomes did prove to be supervital on a retest).

The strains carrying the chromosomes from each locality were then intercrossed in all combinations. With ten chromosomes, 45 combinations are evidently possible. With two localities, there are 90 intrapopulation combinations of chromosomes. The chromosomes from different localities were also tested in all combinations; 100 such interpopulation combinations are possible. Females carrying every one of the 190 combinations of the wild chromosomes were then outcrossed to males homozygous for a certain recessive marker gene. In the progeny of each outcross, ten males were taken at random, i.e., a total of 1900 males. Each male evidently carried one chromosome with the recessive mutant marker, and one wild chromosome which could be either a product of crossing over between the

wild chromosomes in the mother or be similar to one of these wild chromosomes. The males were, then, outcrossed to females with other markers (see below for specifications), and in one more generation cultures were obtained in which flies homozygous and heterozygous for a given chromosome were produced in the same culture. In each culture exactly 100 flies were counted, and the numbers of the homozygotes and heterozygotes scored. For 1900 chromosomes tested, this means 190,000 flies classified and counted.

The only additional experiments made were retests of the 20 original chromosomes for their effects in homozygotes. These retests were made when the tests of the viability effects of the recombination chromosomes were nearing completion. This was considered necessary because of the possibility that these original chromosomes may have acquired lethal or other viability effects by mutation during the time elapsed between their isolation from the wild and the completion of the experiments. The retests were made using the same series of crosses as for the recombination chromosomes, and each of the original chromosomes was tested in six replicate cultures, exactly 100 flies being counted and classified from each culture. With 20 chromosomes, this means 120 cultures and 12,000 flies. Apart from the desirable control, these retests have yielded information on the variance of the results produced by environmental variations in replicate cultures, as well as the possible variance coming from mutations that could have arisen in the stock cultures of the original chromosomes (see below).

Material and technique in Drosophila pseudoobscura

One population sample was collected in the vicinity of Austin, Texas, in March-April of 1953 and the other at Mather, California, in June-August of 1954. From the Texas sample, 178 second chromosomes were taken and analyzed for viability effects when homozygous. The results of this analysis have been published in detail by DOBZHANSKY, PAVLOVSKY, SPASSKY, and SPASSKY (1955, see especially Tables 1 and 2 and the Appendix). A roughly similar sample of California chromosomes was analyzed, with results similar to those described earlier by DOBZHANSKY and SPASSKY (1953).

The experimental technique was described and illustrated in DOBZHANSKY (1946) and DOBZHANSKY and SPASSKY (1953), and can be summarized briefly as follows. Males, or sons of the females collected in nature, are crossed to females homozygous for the recessive mutant glass (*gl*) in the second chromosome. A *single* male from the progeny of each cross is selected and crossed to females in which one of the second chromosomes carries glass, the dominant Bare (*Ba*), and an inversion which suppresses crossing over. In the next generation Bare, nonglass females and males are selected and inbred. Their progenies should consist of 33.3 percent of non-Bare flies, carrying in duplicate the wild second chromosome under analysis, and 66.7 percent of Bare flies (the homozygotes for the *Ba gl* Inversion chromosome mostly die, and the few survivors of this class are not included in the counts). In reality, the proportions of the non-Bare flies vary from zero (when the wild chromosome is lethal in homozygotes), to about 33 percent (normal viability) and even higher (supervital chromosomes).

As indicated above, the ten second chromosomes extracted from each sample for the experiments to be described in the present communication were subvital to normal when homozygous. They were kept in homozygous condition, transferred when necessary to fresh cultures, with five to perhaps 20 pairs of parents used for the transfers. The intercrosses were made in the usual manner, virgin females from one strain being crossed to males from another. A single heterozygous female was taken from each progeny, and outcrossed to homozygous *gl* males. In the next generation, ten males, sons of a single mother, were, as indicated above, taken from each cross, and crossed further, in separate cultures, to females which carried the *Ba gl* Inversion chromosome. These crosses yielded progenies consisting of **Ba**, **Ba gl**, and wild type flies. The **Ba** females and males were selected and inbred, and in the next generation, as stated above, 100 flies were counted in each progeny. These counts constitute the primary data, an analysis of which will occupy our attention in the pages that follow.

All the cultures in which counts were made were raised at 25° C.

Viability in intrapopulational crosses

The data on the effects of recombination of the ten chromosomes of Texas origin are presented in Table 1, and those for chromosomes of California origin in Table 2. Both tables are constructed in the following manner.

The numbers in the open squares on the top and on the left of the tables are the ordinal numbers of the chromosomes studied (i.e., Texas–1, Texas–14, etc., to Texas–121). Each of the squares in the diagonal row bounded by fatter margins contains two figures. The upper figure (Roman type) shows the mean percentage of wild type flies in the six replicate cultures testing the viability in homozygous condition of the corresponding original chromosome. If the homozygotes for a given chromosome possessed a normal viability, the test cultures would yield about 34.20 ± 0.26 percent wild type flies (see Table 1 in DOBZHANSKY, PAVLOVSKY, SPASSKY, and SPASSKY 1955). Among the 20 original chromosomes in our material, only Texas–121 (Table 1) and California–15 Table 2) produced percentages in excess of the standard value; these chromosomes are, then, normal to supervital in homozygotes. The other 18 chromosomes yielded values significantly below 34.2 percent; they are, accordingly, subvital to subnormal when homozygous. The lowest viability in homozygotes is produced by Texas–50, Texas–81, California–25 and California–28 (21.5, 25.3, 22.7, and 25.3 percent respectively). The mean proportion of homozygotes in the test cultures is 27.96 percent for Texas and 28.96 percent for California. This does not mean that Texan chromosomes are more deleterious when homozygous than those from California. These chromosomes are not a random sample of the chromosomes from either region.

The squares to the right and above the diagonal row contain Roman type numerals. These numerals are average percentages of wild type flies in the ten cultures testing the viabilities of the recombination products between a given pair of original chromosomes. Thus, the ten recombination products between the

TABLE 1

Viability (Roman numerals above and to the right of the diagonal line) and variance (Italic numerals below and to the left of the diagonal) of the recombination products of ten original chromosomes (nos. 1, 14, 16 . . . 121) from Texas. $\overline{M}$ and $\overline{V}$— mean viability and mean variance of the recombination products of the crosses in which a given original chromosome is a participant. The squares along the diagonal show the viability (Roman) and the variance (italic) of the original chromosomes. Further explanation in text

	1	14	16	39	50	53	57	81	106	121	$\overline{M}$
1	27.7 *14.3*	30.4	13.6	23.9	27.0	18.4	21.0	25.3	23.8	25.6	*23.2*
14	*64.4*	27.8 *3.3*	25.8	21.5	31.5	27.0	30.8	29.7	18.4	23.9	*26.6*
16	*211.8*	*41.3*	26.2 *-11.5*	16.7	26.1	27.9	26.1	26.7	20.4	16.7	*22.2*
39	*184.6*	*110.1*	*118.8*	30.2 *24.3*	26.6	24.6	34.1	22.9	11.3	11.9	*21.5*
50	*95.0*	*32.9*	*104.8*	*-11.7*	21.5 *21.0*	28.2	30.8	31.3	22.5	21.3	*27.3*
53	*175.3*	*43.2*	*68.2*	*61.5*	*17.7*	29.2 *5.5*	30.0	22.5	27.3	29.5	*26.2*
57	*144.7*	*38.2*	*48.8*	*50.1*	*-0.5*	*-4.6*	28.3 *5.2*	33.3	22.7	14.8	*29.9*
81	*25.3*	*-1.3*	*62.4*	*56.2*	*6.1*	*0.2*	*14.0*	25.3 *-3.8*	23.7	25.8	*26.8*
106	*33.2*	*134.4*	*138.5*	*76.0*	*142.0*	*42.4*	*115.9*	*31.3*	27.7 *31.9*	13.3	*20.3*
121	*146.9*	*41.7*	*99.4*	*59.8*	*63.7*	*-2.1*	*124.0*	*66.4*	*96.5*	35.7 *-11.5*	*20.3*
$\overline{V}$	120.1	56.1	99.3	78.4	50.0	44.6	59.0	29.0	90.0	77.4	

chromosomes Texas–1 and Texas–57 (Table 1) have yielded on the average 21.0 percent of wild type homozygotes. The italic numerals in the rightmost columns in Tables 1 and 2, marked $\overline{M}$, give the mean percentages of homozygotes in the tests of the viabilities of the recombination products of a given original chromosome with the other nine chromosomes from the same locality. Each $\overline{M}$ is, therefore, based on examination of 90 cultures and 9000 flies. Inspection shows that, despite many variations, the mean viability of the recombination products is lower than that of the chromosomes from which they are derived. The grand

TABLE 2

Viability and variance of the recombination products of ten original chromosomes from California. For further details see the legend to Table 1 and text

	3	4	9	11	15	18	19	21	25	28	$\bar{M}$
3	**29.0** *5.4*	26.2	29.1	23.6	26.0	27.4	24.3	18.7	25.2	25.2	***25.1***
4	*43.5*	**30.2** *-1.3*	23.5	23.1	30.8	26.3	9.5	9.0	16.5	9.8	***19.5***
9	*1.7*	*10.7*	**28.2** *4.3*	18.0	29.7	20.5	28.8	20.7	26.4	22.7	***24.4***
11	*25.8*	*6.1*	*77.9*	**27.3** *9.6*	25.0	8.3	23.3	21.8	18.6	16.4	***19.8***
15	*20.1*	*18.4*	*42.2*	*37.0*	**35.2** *-15.4*	21.8	28.0	17.3	27.1	25.3	***25.7***
18	*98.8*	*-0.2*	*66.6*	*47.5*	*24.8*	**29.5** *1.1*	14.7	8.4	31.1	20.0	***19.9***
19	*78.3*	*142.6*	*17.2*	*81.2*	*51.0*	*15.5*	**31.5** *-5.7*	22.6	26.1	12.9	***21.1***
21	*89.7*	*203.8*	*64.3*	*50.7*	*112.2*	*49.0*	*26.1*	**30.7** *-0.6*	21.8	17.1	***17.5***
25	*18.9*	*230.5*	*-8.3*	*155.8*	*66.1*	*-0.7*	*42.1*	*62.7*	**22.7** *-6.1*	21.7	***23.8***
28	*60.9*	*137.8*	*31.1*	*58.6*	*54.7*	*138.4*	*57.8*	*37.0*	*67.5*	**25.3** *11.0*	***19.0***
$\bar{V}$	48.6	88.1	33.7	60.1	47.4	48.9	56.9	77.3	70.5	71.5	

mean for the recombination products of Texas chromosomes is 24.43 percent, and of California chromosomes 21.58 percent. These means are significantly lower than the means for the orginal chromosomes.

This result may seem puzzling. The crossover products are less viable than the chromosomes from which they were derived. The solution of the puzzle is, however, simple. Reference has been made above to the fact that the original chromosomes are not a random sample of the chromosomes in the population; their viability in homozygotes is higher than the mean viability of a random sample of second chromosomes of *D. pseudoobscura* (which is 19.82 percent, see Table 5 in DOBZHANSKY and SPASSKY 1953). The recombination released by crossing over tends to restore the viability distribution which is found among the chromosomes in a natural population. This is what is to be expected if the viability of the homozygotes for the chromosomes taken from nature is a product of epistatic interactions of the polygenes which they contain, rather than an additive product of these polygenes.

Viability in interpopulational crosses

The data on the viability of the homozygotes for the recombination products between Texas and California chromosomes are summarized in Table 3. The Roman numerals in Table 3, like those in Tables 1 and 2, are the mean percentages of wild type individuals in the ten cultures testing the recombination products of a given pair of chromosomes. The italic numerals in the horizontal and vertical rows marked $\overline{M}$ are the means for all the tests in which a given chromosome is involved. Each $\overline{M}$ is based on 100 crosses and, therefore, on 10,000 flies. The grand mean (based, then, on tests of 1,000 chromosomes and 100,000 flies) turns out to be 23.06 percent.

This figure may be compared with the grand means for the intrapopulational crosses described above—24.43 percent for Texas and 21.58 percent for California.

TABLE 3

Viability of the recombination products of chromosomes from Texas and from California. $\overline{M}$*—the mean viability of the recombination products of the crosses in which a given original chromosome is a participant*

TEXAS	CALIFORNIA 3	4	9	11	15	18	19	21	25	28	$\overline{M}$
1	26.2	19.2	20.8	25.1	27.9	29.4	9.6	13.1	22.3	14.4	*20.8*
14	28.9	29.1	31.1	24.2	34.5	24.2	22.7	19.1	31.2	22.7	*26.8*
16	25.9	26.5	22.1	9.6	23.2	21.6	17.5	18.9	23.3	24.8	*21.3*
39	19.7	25.5	27.2	24.7	31.2	23.8	16.2	19.5	24.0	22.8	*23.5*
50	24.4	15.5	23.7	18.1	19.7	28.6	28.2	18.2	32.5	13.1	*22.2*
53	23.7	28.5	26.5	29.8	28.5	22.7	22.3	22.8	29.2	29.5	*26.4*
57	17.3	21.0	23.4	20.4	29.3	15.5	27.0	22.2	27.9	22.3	*22.6*
81	27.3	29.4	20.6	24.1	29.5	18.3	28.3	23.5	26.4	25.9	*25.3*
106	25.0	18.3	20.6	16.0	27.4	18.0	13.3	15.2	19.9	18.8	*19.2*
121	22.1	28.3	23.2	15.4	32.5	9.9	17.6	28.3	24.9	22.5	*22.5*
$\overline{M}$	*24.0*	*24.1*	*23.9*	*20.7*	*28.4*	*21.2*	*20.3*	*20.1*	*26.2*	*21.7*	

The average for the intrapopulational crosses, 23.00 percent, almost exactly matches the 23.06 percent for the interpopulational ones. Our data thus disclose no difference between the viabilities in homozygous condition of the recombination products of chromosomes of similar or of different geographic origin.

Variance in the intrapopulational crosses

As stated above, we have examined the viability in homozygous condition of ten recombination products between every pair of original chromosomes. We have also measured the viability of the homozygotes for the original chromosomes themselves, by testing six chromosomes from the strains in which those original chromosomes were perpetuated. Variances were computed for each group of ten cultures with recombination chromosomes, and for each group of six cultures with the original chromosomes. The variance is, of course, the sum of squared deviations from the mean for each group, divided by nine for the recombination chromosomes, and by five for the original chromosomes.

The analysis of variance will be discussed below. For the time being it will suffice to mention that the observed crude variances, U, have three components. First, there is the binomial sampling error, the magnitude of which is easily computed—it is the product of the numbers of Bare and wild type flies observed in each test, divided by 100 (the number of the flies counted). Subtracting this sampling error from the crude variance, U, we obtain the residual variance, V. This is shown in Tables 1 and 2 by italic numerals in the squares along the diagonal (for the tests of the original chromosomes), and below and to the left of this diagonal (for the tests of the recombination chromosomes).

A word of explanation is needed for the fact that some of the V values in Tables 1 and 2 are negative. This means only that the observed crude variance, U, was in these cases smaller than the binomial variance. In the absence of any other components of variance this would happen about half of the time. Most of the V values are, however, positive.

The mean variance for the original chromosomes (i.e., the average of the italic numerals in the squares along the diagonal in Tables 1 and 2) is 7.85 for Texas and 2.36 for California chromosomes. This variance arises from two sources, namely the environmental differences between different cultures, and the possible genetic diversity which could have arisen in the stock cultures of the original chromosomes while the experiments were in progress (to be discussed).

The variances of the viabilities observed among the recombination chromosomes are much higher than those for repeated testings of the original chromosomes. This is seen quite clearly if we examine the figures in the rows marked $\overline{V}$ at the bottom of Tables 1 and 2. The $\overline{V}$ are the mean variances for the 90 recombination products tested for each original chromosome. They range from 29.0 (chromosome T–81 in Table 1) to 120.1 (chromosome T–1 in Table 1), the grand means being 70.3 for Texas and 60.3 for California chromosomes. The obvious conclusion is that the recombination of the gene contents of the chromosomes from the same Mendelian population releases considerable genetic variance.

Variance in the interpopulational crosses

Crossing over between chromosomes derived from different populations results also in release of a great deal of genetic variance, although not significantly greater than that between chromosomes from the same population. The relevant data are summarized in Table 4. Each figure shown in this table in italic numerals is based on ten recombination chromosomes. Each figure in the marginal rows, marked $\overline{V}$, is an average for 100 recombination products of a given chromosome from one population with ten chromosomes from a different population. The grand mean for the interpopulational crosses (based, then, on tests of 1000 recombination chromosomes) is 62.7.

TABLE 4

Variance of the viabilities of the recombination products of chromosomes from Texas and from California. $\overline{V}$*—mean variance in the crosses in which a given original chromosome participates*

		CALIFORNIA										
		3	4	9	11	15	18	19	21	25	28	$\overline{V}$
TEXAS	1	122.6	136.7	33.3	6.0	109.8	-7.8	148.3	148.7	152.2	119.0	96.9
	14	-7.8	26.6	-2.7	76.1	40.1	22.3	127.1	82.2	-5.5	13.8	37.2
	16	26.9	5.5	63.8	74.7	113.2	33.3	52.5	56.6	32.1	24.9	48.3
	39	51.3	6.6	-0.5	8.3	17.4	31.6	121.9	111.7	38.2	59.5	44.6
	50	0	270.7	53.3	31.7	193.8	30.1	19.9	71.7	33.7	139.8	84.5
	53	17.9	28.8	9.5	6.4	-2.8	76.0	20.0	83.5	75.3	39.9	35.4
	57	39.3	137.2	84.4	50.9	18.9	105.8	51.2	90.7	29.8	37.6	64.6
	81	23.7	-13.2	138.6	7.8	-0.3	42.6	95.0	100.7	39.1	163.8	59.8
	106	12.6	179.3	85.9	91.2	30.6	63.9	88.0	42.4	196.8	132.0	92.3
	121	86.9	9.7	51.9	71.5	50.8	98.7	214.9	13.0	17.4	20.6	63.5
	$\overline{V}$	37.3	78.8	51.7	42.5	57.1	49.7	93.9	80.1	60.9	75.1	62.71

Release of variability

The quantitative aspects of the release of variability by crossing over will now be considered. The basic question is the magnitude of the "real variance", V.

For the original Texas chromosomes, $\overline{U}$, the mean of the observed variances between replicate cultures, is 27.87. The mean viability, $\overline{M}$, is 27.95 percent, giving a theoretical binomial variance of 20.14 and a "real variance" of $27.87 - 20.14 = 7.73$. This differs by only a negligible amount, as it must, from the mean of individual V's, $\overline{V} = 7.85 \pm 5.6$, and for convenience we will use the quantities $\overline{V}$ hereafter. It may be noted that values of V were obtained for the same chromosomes in other tests more than a year earlier (DOBZHANSKY and LEVENE 1955), with $\overline{V} = 13.9$. This is excellent agreement, since experimental conditions for the earlier experiment were somewhat different and some mutational changes may have occurred in these chromosomes during the interim.

For the original California chromosomes the values were $\overline{U} = 20.70$, $\overline{X} = 28.95$, $\overline{V} = 0.21 \pm 4.1$. The values of $\overline{V}$ for Texas and California do not differ significantly from each other, and neither one is significantly different from zero although that for Texas is close to significance.

A quite different picture appears when the recombination chromosomes are studied. This is true for recombination chromosomes resulting from Texas intrapopulational crosses ("Texas intra") $\overline{V} = 70.3 \pm 8$, for recombination chromosomes resulting from California intrapopulational crosses ("California intra") $\overline{V} = 60.3 \pm 8$, and for recombination chromosomes resulting from interpopulational crosses between a Texas chromosome and a California chromosome ("inter") $\overline{V} = 62.7 \pm 6$. These values do not differ significantly among themselves, but all are very significantly greater than those for the original chromosomes. This excess is evidently due to differences in the viability, when homozygous, of different recombinant chromosomes from the same two original parent chromosomes; that is, to the release of genetic variability by recombination between normal or quasinormal chromosomes.

A more intuitive feeling for the magnitude of the variability released by recombination may be given as follows. For the original chromosomes the standard deviation of the true viability between replicate cultures ($\sqrt{\overline{V}}$) is between zero and four with two to three as the most likely value. This means that most cultures would have viabilities differing from the mean viability for that particular chromosome by not more than two standard deviations, six. Since the grand mean viability for the original chromosomes was 28.5, most cultures are quasinormal or normal, with few supervital. On the other hand, for the recombinant chromosomes the standard deviation between replicate cultures (involving different recombinants from the same parent chromosomes) is close to eight, implying that most recombinants differ from the mean for that cross by anywhere from zero to 16 percentage points. Thus the range within replicate cultures where recombination can occur is perhaps three times as great as when it cannot. Furthermore we know that chromosomes that are supervital when homozygous are seldom found; yet they should be found in nature with at least the frequency at which they occur

by recombination. Hence the range of ± 16 for the recombinants should include few values above, say 34, which means that the central value should be perhaps 18 to 20. Actually, the distribution of viabilities is not symmetric, and there are many cultures whose viability is zero or very small. Hence instead of ± 16, it might be better to say something like − 22, + 12 with more cultures on the high side. In point of fact, this is just about what we find; the grand mean viability for recombinant chromosomes is 23, with individual cultures going from zero to the 30's. This, of course, is for observed values, including binomial sampling variance. All of this is simply another and more quantitative way of saying what was said above; that is, starting with selected chromosomes with above average viability, recombination is more likely to spoil them than to improve them—they regress toward the mean.

Analysis of variance for means

The present data were collected in such a way as to facilitate the use of the analysis of variance. There are five experiments—two on the original chromosomes, two on the recombination chromosomes from intrapopulational crosses, and one on the interpopulational crosses. The results of the analysis of variance are summarized in Tables 5 and 6.

The controls form a one way classification with equal numbers (6) of replicates. The mean square between chromosomes measures the difference of viability of the chromosomes used, and since they are a selected set it is of little immediate interest. The mean square within chromosomes is $\overline{U}$, and when the binomial component is subtracted becomes $\overline{V}$.

TABLE 5

Analyses of variance

Description	d.f.	On means F	P, %	On variances F	P, %
		Texas intrapopulational crosses			
Main effect	9, 35	4.33	0.05– 0.1	3.72	0.1– 0.5
Interaction	35, 405	2.22	<0.1	1.09	50
Int. of int.	9, 35	2.74	1 – 2.5	1.48	10 –25
Within	405, 50	3.18	≪0.1	11.5	≪0.1
		California intrapopulational crosses			
Main effect	9, 35	3.27	0.5 – 1	0.78	50 –75
Interaction	35, 405	3.39	≪0.1	2.08	<0.1
Int. of int.	9, 35	4.78	<0.1	3.95	0.1– 0.5
Within	405, 50	3.71	≪0.1	20.2	≪0.1
		Interpopulational crosses			
Texas main	9, 81	2.95	0.1 – 0.5	0.82	50
California main	9, 81	3.74	0.05– 0.1	1.16	25 –50
Interaction	81, 900	2.53	≪0.1	2.1	<0.1
Texas int. of int.	9, 81	1.56	10 –25	1.8	10
Cal. int. of int.	9, 81	1.76	10	1.5	10 –25
Within	900, 100	3.30	≪0.1	13.7	≪0.1

TABLE 6

Components of variance for means, M, and real variances, V. Bold face figures are estimates. Figures above and below them are approximate upper and lower 95 percent confidence limits respectively. Values of within crosses and residual components for V are for comparison only (see text). All values have two significant figures.

Description	Additive between crosses	Nonadditive between crosses	Total between crosses	Within crosses	Total genetic	"Real" residual	Binomial sampling	Total residual
MEANS								
Intra T	33	25	..	77	..	23	21	41
	8.2	**11**	19	**62**	81	**7.8**	**18**	**26**
	2.3	3.9	..	46	..	0	15	18
Intra C	32	37	..	72	..	11	20	29
	7.4	**18**	25	**60**	85	**0.20**	**17**	**17**
	1.3	9.3	..	47	..	0	14	14
Inter T	18	..	..	..	..	..	..	..
	4.0	..	..	..	..	..	..	..
	0.69	..	..	..	..	..	..	..
Inter C	23	20	..	68	..	13	20	30
	5.6	**12**	22	**58**	80	**4.0**	**18**	**22**
	1.5	7.2	..	49	..	0	16	16
Orig T	41	..	..	..	..	23	21	43
	8.5	..	8.5	..	8.5	**7.8**	**20**	**28**
	1.1	..	..	..	..	0	19	19
Orig C	36	..	..	..	..	11	21	32
	8.3	..	8.3	..	8.3	**0.21**	**20**	**21**
	1.8	..	..	..	..	0	19	19
VARIANCES								
Intra T	30	15	..	22	..	..	..	0.38
	7.4	**1.5**	8.9	**16**	25	..	..	**0.15**
	2.0	0	..	11	..	..	..	0.07
Intra C	8.0	33	..	17	..	..	..	0.18
	0.020	**14**	14	**12**	26	..	..	**0.06**
	0	4.5	..	9.0	..	..	..	0.04
Inter T	14	37	..	17	..	..	..	0.20
	1.6	**16**	18	**13**	31	..	..	**0.10**
	0	8.0	..	10	..	..	..	0.06
Inter C	8.4	..	..	..	..	..	..	..
	0.40	..	..	..	..	..	..	..
	0	..	..	..	..	..	..	..
Orig T	4.3	..	..	..	..	..	..	10
	0	..	0	..	0	..	..	**3.1**
	0	..	..	..	..	..	..	1.5
Orig C	0.45	..	..	..	..	..	..	5.7
	0	..	0	..	0	..	..	**1.7**
	0	..	..	..	..	..	..	0.81

The data from the intrapopulation crosses from a triangular classification, with ten replicates in each cell, of the type known as a diallel analysis of variance table. Sums of squares and mean squares were calculated from this, and used for the F tests summarized in Table 5. Finally, from the mean squares, components of variance were obtained and approximate confidence limits for them found. The statistical methods used for all this are given in a paper to appear (Levene 1959); here it is only necessary to discuss the interpretation of the results. For definiteness, we will discuss the results from the Texas intracross in some detail.

The total variability of viability can be separated into the following components: Additive genetic variability, nonadditive genetic variability, genetic variability between different crosses (which is the sum of the previous two components), genetic variability within crosses, total genetic variability (which is the sum of all three genetic components), and residual variability, which further subdivides into variability of the residual genotype, environmental variance, and binomial sampling variance. These components are listed in the usual hierarchical order, but it will be more convenient to explain them in the reverse order.

The binomial sampling variance is $\overline{M}\ (100 - \overline{M})/100 = 18.31$ and has a negligible standard error. The residual real variance due to environment, genetic effects of the other chromosomes, and possible mutant genes on the original chromosomes, cannot be estimated from the data on the recombinant chromosomes, but it can be estimated from data on the original chromosomes, since the "real variance" between replicate cultures of these, estimated by $\overline{V} = 7.8$, involves precisely these factors. The total residual variance for the Texas intra data is then $18.3 + 7.8 = 26.1$, with 95 percent confidence limits 18 and 41. Now if there were no variability due to recombination within crosses, the observed mean square within crosses, $\overline{U}$, would be equal to this residual total variance. Actually, $\overline{U} = 88.4$ and the difference, $88.4 - 26.1 = 62.3$, estimates the amount of variability actually due to crossing over. (The necessary assumption is made that the residual variance due to environment and residual genetic effects is the same for the derived chromosomes as for the original ones—in particular that the derived chromosomes are no less homeostatic when homozygous than the original ones. This may not quite be true; since the derived chromosomes have lower mean viability, they may also be less homeostatic, but if so, two closely related facets of recombinational breakdown of above average chromosomes are being confounded. In any event, the additional environmental variance is probably small compared to the genetic variance.) It should be noted that this variance component for genetic variance within crosses is essentially $\overline{V}$ for the derived chromosomes, minus $\overline{V}$ for the original chromosomes, and thus refines the analysis of the previous section.

We next consider the genetic variance between crosses. The mean viability for a particular cross, M, is the mean of the viabilities of ten cultures. Each of these is subject to the total variance within crosses, $\overline{U}$, including binomial sampl-

ing variance, residual variance, and within cross genetic variance. From these causes alone M has variance $\overline{U}/10$. Any excess of the actual variance of the 45 values of M over this value of $\overline{U}/10$ is due to genetic differences between crosses. The further analysis of this between crosses variance is somewhat simpler in the interpopulational cross, to which we now turn.

The main effect of a particular Texas chromosome, e.g., T1, is the difference between the mean of all recombinant cultures involving T1 and the mean for all cultures, and is $20.80 - 23.06 = -3.76$. Similarly the main effect of a California chromosome; e.g., C3, is the mean of all cultures involving C3 minus the grand mean, or $24.05 - 23.06 = +0.99$. We could then predict that the cross between T1 and C3 would produce a viability equal to the grand mean plus the effect of T1 plus the effect of C3, or $23.06 + (-3.76) + 0.99 = 20.29$. The value actually observed for this cross is 26.2, and the difference between the observed and predicted values is $26.20 - 20.29 = +5.91$. This is called the interaction and is a measure of the nonadditivity of the influence of these two chromosomes. From the sum of the squares of the interactions of all 100 interpopulational crosses, we obtain a mean square for interaction, as shown in Levene (1959). In the present case this mean square is 20.3. As explained above, the mean square for interaction should be equal to $\overline{U}/10$ merely as the result of total within crosses variation; any excess over this figure represents the contribution of nonadditive variance between crosses. For the present data this component of variance is $20.3 - 80.4/10 = 12.3$, with 95 percent confidence limits 7 and 20.

Finally, from the sum of squared deviations of the averages for the ten Texas chromosomes from the grand mean (i.e., the sum of the squared main effects), we obtain a mean square for the main effect of Texas chromosomes, 5.99. Again, since the average for each Texas chromosome is based on ten crosses involving that chromosome, the mean square should be at least one tenth the total interaction mean square. The excess, $5.99 - 20.3/10 = 3.96$ with 95 percent confidence limits 0.7 and 18, is an estimate of the variation in the consistent effects of different chromosomes; that is, it is the additive component of variance between crosses. Similarly the additive component of variance between California chromosomes is 5.6 with 95 percent confidence limits 1.5 and 23. Adding the two additive components and one nonadditive component of variance we obtain $5.6 + 4.0 + 12.3 = 21.9$ for the genetic variance between crosses.

In the more customary use of an analysis where all intercrosses are made and then some property of the heterozygotes is studied, the additive component is often called the variance of general combining ability, and the nonadditive component is called the variance of specific combining ability. In the present study of the properties of homozygous recombination chromosomes, these terms are misleading. The special nature of the present experiment also has an interesting consequence. From the point of view of statistical theory any of the components of variance we have discussed can be positive (i.e., exist) without implying anything about the others. However, the genetics of this situation ensures that if there are any differences between chromosomes, segregation and crossing over

will create differences in sister recombination chromosomes. Thus a significant variance between crosses implies a within crosses genetic component, although the reverse is not true.

Analysis of variance for variances

In the discussion of the preceding section it was tacitly assumed that the true variance within crosses was constant and that the observed values differed only because of sampling errors. If this is not true, that discussion is still essentially correct, with the understanding that by the variance within crosses the mean variance is meant. However, the variation of the "real variances" within crosses, V, is of interest and can itself be analyzed by the analysis of variance. This time, however, each cross gives only a single value of V, while it gave ten values of M. The additive component of variance is obtained just as before, but a complication arises in getting the nonadditive component of variance between crosses. It is simple to obtain the mean square for interaction, but this only implies real nonadditive differences between crosses if it is bigger than would be expected from the total variance within crosses, and we cannot get a variance from a single number. Luckily statistical theory can obtain a theoretical sampling variance within crosses in the form $2\overline{U}^2/9$ (LEVENE 1959). Thus the components of variance for V can be obtained and are given in Table 6. It should be noted that only the components of variance between crosses are meaningful. The others are derived from the entries in the components of variance table for M, and give no new information. They are listed simply as a yardstick for evaluating the size of the components between crosses.

Results of analysis of variance

The F statistics for testing all the components of variance, and the corresponding P values, are given in Table 5, while the components themselves, with very approximate confidence limits (LEVENE 1959) are given in Table 6. For uniformity, all values in Table 6 are given two significant figures. In no case is more than two justified, while in some cases the second figure is of doubtful value. It will be noted that the confidence intervals for the between crosses and "real" residual variance components are disappointingly large; those for within crosses and binomial sampling variance of the means are fairly good. Nevertheless the consistency of the results suggests taking the data on its face value for the purposes of discussion, rather than taking the pessimistic point of view implicit in demanding 95 percent confidence limits.

Considering first the analyses of the data on means, the additive between crosses component is always significant, and varies from 4.0 to 8.2, compared to 8.3 and 8.5 for the original chromosomes. This suggests that the consistent (additive) effects of particular parent chromosomes on their crossover products may be simply a reflection of their original differences in viability, with "good" chromosomes giving good recombination products. However, when the mean value for recombinants involving given chromosomes are compared with the mean for

the original chromosomes this is seen not to be so. Correlation coefficients can be calculated for all comparisons of the ten chromosomes under two different conditions. These are original, intra, and inter, and, for Texas, the result of the previous study under different environmental conditions. These correlations are given in Table 7. These coefficients are subject to considerable sampling error, although its proper magnitude is not at all clear (LEVENE 1959). However it is plain that there is little or no relation between the value for a given chromosome and the value of its recombination products. On the other hand, there is some indication that chromosomes that give good recombination products in intrapopulational crosses tend to do so also in interpopulational crosses.

As might be expected from the above analysis, the nonadditive component of variance between crosses is somewhat larger than the additive one, but not significantly so. Furthermore the component of variance within crosses is substantially (and significantly) larger than that between crosses, again indicating the unpredictability of the viability of a given crossover chromosome. In this connection it should be noted that normally nonadditivity is due to dominance and epistasis and it is difficult to untangle the two. Here all chromosomes are tested in homozygous condition, so only epistasis is involved. It is not possible directly to assess the additive and epistatic components of the variance within crosses. However, there must be a substantial epistatic component, first because there is epistatic interaction variance between crosses, and second because of the decline in mean viability of recombination chromosomes.

Finally the "real" residual variance, made up of environmental and residual genetic variance, which is known only for the original chromosomes, is small in comparison to the total genetic variance. Its order of magnitude is in agreement with that previously found for homozygous chromosomes (DOBZHANSKY and LEVENE 1955).

Turning to the components of variance for the variances, V, the additive component is significant only for the Texas intra data, while the nonadditive component is significant for the other two crosses. Thus in each case there is a significant variance between crosses. The order of magnitude of this between crosses component is about the same as the "within component", which is not observable directly but can be inferred on theoretical grounds. This means that about half of the observed variation in V between crosses is explainable as random sampling error for normally distributed data, but the other half is due to real variability in the amount of variability released in different crosses (or, possibly in part, to nonnormality of the data). It is hardly surprising that there should be such variability, since some pairs of chromosomes will surely differ more in their gene contents than will others; it is rather surprising that this variability should be so small.

So far we have not mentioned the lines in Table 5 labeled "Int. of int.." that is, interaction of interactions. This test was to investigate the possibility that the nonadditive component of variance would be more important when certain chromosomes were involved as one of the partners in a cross. A significant value

would mean that differences in the mean square interaction for different chromosomes were greater than could be expected by chance, or in other words some chromosomes were more predictable than others. This might happen even when the average interaction for all chromosomes was not significant. In point of fact the interaction of interaction was never significant unless the ordinary interaction was, and might or might not be significant when the ordinary interaction was significant. Nevertheless in at least a few cases there was a real difference in the amount of unpredictability introduced by different chromosomes.

Finally it should be mentioned that neither for the means of M and V nor for the components of variance of M and V, were there any significant differences between Texas and California, or between intra- and interpopulational crosses. The differences between the original and recombination chromosomes have already been discussed.

Lethal recombination chromosomes

Some of the recombination chromosomes, both in the intra- and in the interpopulational crosses, were lethal in double dose. A list of the crosses which produced such lethals, with numbers of the lethal chromosomes in each cross which yielded them, is given in Table 8. A test culture which contained a lethal chromosome produced, of course, 100 **Ba** flies and no wild type flies. Table 8 shows also that mean viability (the mean percentages of wild type flies) of the recombination chromosomes which did *not* act as lethals, and the variances of these viabilities. These figures may be compared with those in Tables 1–4, which show viabilities and variances of recombination chromosomes *including* the lethals.

In all, 44 out of 900 recombination chromosomes in the intrapopulational crosses, and 33 out of 1000 chromosomes in the interpopulational ones, contained lethals, or 4.9 percent and 3.3 percent respectively. The numbers of crosses which produced at least one lethal chromosome were 19 in the intrapopulational and 19 in the interpopulational crosses. None of these differences are significant. If all the crosses are combined, we have the frequencies 4.1 percent lethals among the 1900 chromosomes tested, and 20 percent of the crosses giving at least one lethal recombination product.

TABLE 7

Correlation between the means in different experiments for Texas (above the diagonal) and California (below the diagonal) chromosomes

	Original chromosomes	Intra-populational	Inter-populational
Previous experiment	—0.42	0.09	—0.75
Original chromosomes		—0.58	0.05
Intrapopulational	0.11		0.61
Interpopulational	0.11	0.79	

The question that immediately arises is whether all chromosomes are equally liable to yield lethal recombinants, or whether some of the original chromosomes are more prone to do so than others. Consulting Table 8 we find that the latter alternative is correct. In the nine intralocality crosses in which it participated, the chromosome C-4 has yielded 20 lethals among 90 recombination chromosomes tested, while the chromosomes C-9 and C-15 yielded no lethals. In the interlocality crosses, C-4 gave nine lethals among the 100 recombination chromosomes tested, while C-3 gave none in a like number of tests. Chi-square tests for heterogeneity among California and Texas chromosomes in intralocality and interlocality crosses all show highly significant heterogeneities. At the same time, some of the original chromosomes which yielded many lethals in the intrapopulational crosses gave few in the interpopulational ones, and vice versa. Thus, the chromosome T-50 gave 11 lethals in the interpopulational and two in the interpopulational crosses.

The recombination chromosomes which acted as lethals when homozygous could have arisen in either or both of the following ways. First, the original chromosomes may have contained gene complexes which, though not lethal in themselves, could yield homozygous lethal compounds when combined with genes from other chromosomes. These are synthetic lethals. Second, single, dis-

TABLE 8

Numbers of synthetic lethal chromosomes obtained in different crosses, and means and variances of viability (percentages of wild type flies), in cultures containing nonlethal chromosomes
T = Texas, C = California

Cross	Lethals	Viability		Cross	Lethals	Viability	
		Mean	Variance			Mean	Variance
T1 × T16	4	22.67	138.4	T1 × C4	2	24.00	45.8
T1 × T39	1	26.56	129.3	T1 × C19	3	13.71	127.7
T1 × T53	1	20.44	150.8	T1 × C21	1	14.56	143.8
T1 × T121	1	28.44	75.4	T14 × C21	1	21.22	42.5
T14 × T106	1	20.44	104.8	T16 × C11	1	10.67	71.5
T16 × T39	1	18.56	95.4	T50 × C4	5	30.100	16.6
T39 × T106	1	12.56	68.0	T50 × C15	3	28.14	16.9
T39 × T121	2	14.88	27.2	T50 × C28	3	18.71	89.0
T50 × T106	2	28.13	3.9	T53 × C21	1	25.33	22.6
T57 × T121	1	18.50	82.4	T57 × C4	1	23.33	93.9
T106 × T121	2	16.63	61.8	T57 × C9	1	26.00	19.8
C3 × C18	1	30.44	10.2	T57 × C18	1	17.22	86.2
C3 × C21	1	20.78	53.0	T106 × C4	1	20.33	155.8
C4 × C19	5	19.00	99.1	T106 × C9	1	22.89	38.5
C4 × C21	7	30.00	–12.0	T106 × C19	2	16.63	51.0
C4 × C25	3	23.57	153.9	T106 × C25	1	22.11	164.1
C4 × C28	5	19.60	74.0	T106 × C98	1	20.89	100.1
C11 × C18	3	11.86	23.0	T121 × C18	3	14.14	79.3
C18 × C21	2	10.50	38.3	T121 × C19	1	19.56	199.3

crete mutant genes lethal when homozygous may have arisen in some of the chromosomes owing to point mutations.

Synthetic lethals

It will now be shown that the assumption that all the observed lethals are the result of point mutations leads to consequences that can be ruled out statistically.

As shown in Table 8, 21 out of the 190 crosses, or 0.1105 of them, gave one lethal chromosome out of ten tested. If the female heterozygous for the two original chromosomes was heterozygous for a lethal as a result of a point mutation, each of her sons would have an independent probability of 0.5 of carrying this lethal. The probability that exactly one son out of ten carried it, giving one lethal-containing culture, is 10/1024 or about 0.01. We would then expect two such crosses out of 190, even if all the females carried a lethal (and in fact 152 of the 190 crosses gave no lethals). Therefore, on the assumption that all the lethals arose by point mutations, it is safe to suppose that nearly all the 21 crosses with one lethal culture are the result of mutation affecting the heterozygous male grandparent of that culture.

Now let p be the probability of such a mutation; then $10\,p = z$, is the expected number of lethal cultures per cross, and the actual number of lethal cultures will behave like a Poisson variable. The probability of one lethal is ze^{-z}, with an observed value of 0.1105, giving an estimate of $Z = 0.125$. Then from mutation at this stage we would expect 167.7 crosses with no lethals, 21.0 with one lethal, 1.3 with two lethals, and 0.05 with more than two lethals. If we take the upper 95 percent confidence limits for Z of about 0.2, we would expect only 3.11 crosses with two lethals, 0.20 crosses with three lethals and 0.01 crosses with four or more lethals. Thus at most one of the cultures with three lethals and none of those with more than three lethals could be due to mutation at this level. The remaining crosses with three or more lethals would then derive from females heterozygous for a lethal. There are ten of these: five with three lethals, one with four, three with five, and one with seven. We can compare this with what would be expected in ten binomial samples of ten observations with $p = \frac{1}{2}$, taking account of the fact that we have eliminated any samples with zero, one, or two lethals. A chi-square test gives $\chi^2 = 14.9$ with P approximately one percent. This significant value is due almost entirely to the five crosses with three lethals; the exact probability of this alone is 0.004. On the other hand if, as is much more likely, none of the crosses with three lethals result from three independent mutations, there are six such crosses and χ^2 becomes 19.6, with $P =$ 0.2 percent. Thus either an improbable event has occurred, following by another one significant at the one percent level, or we have significance at the 0.2 percent level or better.

It is thus clear that the distribution of the number of lethal cultures between different crosses cannot reasonably be explained on the assumption that they are all due to point mutation. Thus, some, at least, of them must be synthetic lethals.

Distribution of synthetic and point lethals

An attempt may now be made to estimate at least roughly what proportions of the lethal recombination chromosomes in our material contained synthetic and point lethals respectively.

The recombination chromosomes in our material may have acquired a lethal point mutation in one of two ways. First, such mutations may have arisen during the approximately one year which had elapsed between the times when the original chromosomes were tested and found to give viable homozygotes and the conclusion of the experiments. Secondly, they may have arisen in some of the eggs of the females heterozygous for pairs of original chromosomes in which the recombination was allowed to occur.

The spontaneous mutation rate for lethals in the second chromosome of *D. pseudoobscura*, in cultures kept at room temperature (about 22°-23° C), was found by DOBZHANSKY and SPASSKY (1953) to be 0.56 ± 0.12 percent per generation. For our purposes this value may be taken as the upper limit, since the stock cultures of the original chromosomes were kept at 16° C, and since it is well known that lowering the temperature results, in Drosophila, in considerable drop of the spontaneous mutation rates. Now, among the 1900 recombination chromosomes tested in our experiments one may expect on this basis approximately 11 lethals appearing singly among the ten chromosomes tested from a given cross (ignoring the improbable coincidence of two independent mutations among ten chromosomes, and ignoring the possible bunching of the mutants as a result of mutation in early developmental stages). We actually have 21 single lethals (Table 8).

Furthermore since the stock cultures of the original chromosomes were kept for a year between the tests, some of the flies in these stocks may have come to be heterozygous for lethal mutants. At 16° C the stocks were transferred at about six-week intervals, so that during a year roughly ten generations have elapsed. Taking the mean age of the cultures when recombination of the chromosomes occurred to be five generations, about $0.56 \times 5 = 2.8$ percent of the chromosomes in these cultures might have carried discrete lethals that arose by point mutations. That this is an overgenerous estimate can be shown in two ways. First, six chromosomes from each culture of the 20 original chromosomes were retested at the end of the experiments, and none of these 120 chromosomes proved to be lethal when homozygous, while $120 \times 0.56 = 6.7$ lethal chromosomes could have been expected. Secondly, as shown in the foregoing section, the lethals which were carried in the females in which the recombination occurred should have appeared in about half of the ten resulting recombination chromosomes. Actually, as shown in Table 8, only three batches of recombination chromosomes (all involving the chromosome C-4) yielded five lethals, one (likewise involving C-4) yielded seven lethals, and one yielded four lethals. The probability that a female heterozygous for a lethal will transmit it to only one of her ten tested sons is about one percent. Thus, we have too many batches of tested chromosomes with single lethals to be accounted for on this basis.

Very roughly, we may estimate that about half of the lethals in the recombination chromosomes were synthetic and the others were point lethals. To go beyond this, experiments must be made crossing flies with the presumed synthetic lethal chromosomes to a strain having its second chromosome marked with convenient mutant genes, obtaining crossovers, and testing the latter for lethality. With point lethals, one would thus find the locus at which the lethal is situated. With synthetic lethals which depend for their lethal effects on interaction of genes lying far apart in the chromosome, the linkage test will disclose no single locus to which the lethality could be ascribed. In other words, synthetic lethals could be "desynthesized". Experiments of this sort have been started, and their results will be reported elsewhere in due time. Thus far, among ten chromosomes tested, six chromosomes seem to contain synthetic and four chromosomes point lethals. It may also be remarked at this point that the bunches of four, five, and even seven lethals among groups of ten chromosomes tested may consist of synthetic lethals, as will be shown in the article dealing with *D. prosaltans* (to be reported).

Nonlethal cultures

In order to make sure that the general conclusions about the changes in viability were not due primarily to the presence of lethal chromosomes, the previous analysis was repeated on the data with lethals omitted, using the values of M and V given in Table 8. As might be expected, since only 4.1 percent of the 1900 chromosomes tested were eliminated, the main outline is unchanged, although in general the mean is increased and the variability decreased.

The most important new values are as follows. For the Texas intrapopulational cross, $\overline{M} = 25.1$ and $\overline{V} = 56$; components of variance for the means are additive between crosses, 6.2, nonadditive between crosses, 6.5, within crosses, 48. For California the corresponding values are 22.9, 47.8; 4.9, 11, 47. For the interpopulational cross we have $\overline{M} = 23.8$, $\overline{V} = 52.7$; components of variance for the means are Texas additive, 2.7, California additive, 6.2, nonadditive, 14, within crosses, 48. Statistical significance is essentially unchanged.

Since the analyses with and without inclusion of lethals give such similar results, we decided to report only one in detail, and chose the complete data, since lethality is simply a part, though an extreme one, of the picture of variation in viability.

SUMMARY

Ten second chromosomes of *Drosophila pseudoobscura* were taken from the populations of the two localities—one in Texas and the other in California. Individuals which carry these original chromosomes in double dose (homozygously) are normally viable to subvital under the conditions of the experiments.

By means of suitable crosses, chromosomes were then obtained, most of which, owing to the occurrence of crossing over in their mothers, were compounded of sections of the original chromosomes. A total of 1900 such recombination chromosomes were then tested for their viability effect in homozygous condition. Of

these, 900 chromosomes were recombination products of pairs of the original chromosomes from the same locality (90 intrapopulational crosses, ten recombination chromosomes tested from each cross). The other 1000 recombination chromosomes were derived from the 100 interpopulational crosses (i.e., pairs of the original chromosomes from different localities), ten recombination chromosomes per cross.

The viability of the homozygotes for the recombination chromosomes varied all the way from normality to complete lethality. At least some of the lethal recombination chromosomes carried "synthetic lethals", i.e., their lethal effects when homozygous were due to interaction of complexes of polygenes which were not lethal in other combinations.

Statistical analysis has disclosed that a great wealth of genetic variance is released by recombination of the gene contents of the original chromosomes. The recombination products of some of the original chromosomes are more viable than those of others, and some of the original chromosomes release more genetic variance than do others. But the most prominent determinants both of the viability and of the variability of the recombination products reside in the specific interactions of the members of a given pair of the original chromosomes. Thus, a combination of chromosomes A and B may release, through recombination, considerably more (or less) genetic variance than either of these chromosomes does with some third chromosome, C. The viability of the recombination products is, thus, an outcome of epistatic interactions of the genes which these chromosomes carry, rather than a simple additive product of these genes.

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JOINT INTERPRETATION OF HETEROSIS AND GENETIC VARIANCES IN TWO OPEN POLLINATED VARIETIES OF CORN AND THEIR CROSS[1]

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IN past studies, heterosis of yield of the cross of two open pollinated varieties of corn, Jarvis and Indian Chief, was 26 percent and genetic variances within these two varieties were sufficient to allow considerable improvement through selection. A recent investigation of the variances within the cross of the varieties has indicated the variances were much less in the cross than in the parent varieties. The purpose of this manuscript is to jointly interpret the results on heterosis, on the genetic variances within the varieties and on the genetic variances within the cross of the varieties as to their compatibility with the simple genetic model of dominance of favorable genes such as proposed by BRUCE (1910).

MATERIALS AND METHODS

Methods and results for the genetic variances within the varieties were reported by ROBINSON *et al.* (1955), and for the heterosis observed in the cross of these varieties by ROBINSON *et al.* (1956).

In the recent study of the cross between the Jarvis and Indian Chief varieties, a random plant from one variety was mated as a male to four random plants from the other variety. The four half-sib families with a common male parent constituted a male group. The procedure followed was as described by ROBINSON *et al.* (1949), except that male and female parents were from different varieties. Sixty-four male groups were made for each variety. Four male groups, four progenies per male, were randomly chosen to form a block of 16 progenies and were tested with two replications, giving a block of 32 plots. An additional plot in each replication contained one of the two varieties with the parental varieties being represented in different replications of a block. A total of 16 such blocks of material were used within each of the two reciprocally crossed populations. Thus a population consisted of 512 plots of progenies and 16 plots of each of the Jarvis and Indian Chief varieties. The material was tested at two locations in 1954, and a second sample of progenies, prepared in 1954, was tested at the same locations in 1955. Locations were the Central Crops Research Station, Clayton and Upper Coastal Plain Research Station, Rocky Mount. Similar soil types occur at each station situated about 60 miles apart in the Coastal Plain area of North Carolina.

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Plots were two rows wide and ten hills long with three feet between rows and plants spaced 18 inches within rows.

The data were of yield of ear corn and based on ten competitive plants in each plot whenever possible. Except for the variation among plants in plots, analyses were of plot values computed on a mean per plant basis. Individual plant data were taken on approximately every twelfth plot of the cross bred progenies.

The data for each sample of progenies in a population were analyzed by individual blocks and the results pooled to give the combined analysis of an individual test shown in Table 1.

TABLE 1

Variance analysis appropriate for individual tests

Source of variation	d.f.	m.s.	Expectation of m.s.
Blocks	b-1		
Replications in blocks	b(r-1)		
Males in blocks	b(m-1)	M_1	$\sigma^2+r\sigma_f^2+rn\sigma_m^2$
Females in males in blocks	bm(n-1)	M_2	$\sigma^2+r\sigma_f^2$
Remainder among plots	b(mn-1)(r-1)	M_3	$\sigma^2 w/_k+\sigma_e^2=\sigma^2$
Plants in plots	$\Sigma k_i'$-1	M_4	σ_{ic}^2

b = blocks per test.
r = replications per block.
m = male groups in each block.
n = females per male.
k_i' = number of plants in *i*th plot on which individual plant data were taken.
σ_{ic}^2 = sum of intraplot genetic and environmental variance among individuals of the same progeny.
σ_e^2 = interplot variance.
σ_f^2 = variance attributed to female parents mated to the same male.
σ_m^2 = variance attributed to male parents.

The procedure in estimation of the components of variance from this analysis of variance, designated Design I, has been given by COMSTOCK and ROBINSON (1952). The estimates of σ_m^2 and σ_f^2, from the analysis of a single year and location as in Table 1, contain interaction effects of locations and years. For example, the estimate of σ^2 is expected to contain, in addition to the male component, components of variance for the interaction of male parents with years, with locations and with years × locations. Similarly, interaction components are expected to contribute to the estimate of σ_f^2.

RESULTS

Experimental

Yield of Jarvis, Indian Chief and their cross at three locations in 1952 and 1953 have been reported by ROBINSON *et al.* (1956). Additional data for these varieties

and their cross are available from the tests of the two populations of crossbred progenies in 1954 and 1955. The mean yield of the varieties and their cross for the four years are summarized in Table 2.

TABLE 2

Yields of Jarvis, Indian Chief, their cross and the heterosis in the variety cross

Year	Number of locations	Jarvis		Indian Chief		Variety cross		Heterosis	
		Plots	Yield	Plots	Yield	Plots	Yield	Percent	lb/plant
1952	3	15	0.32	15	0.29	30	0.39	29	0.09
1953	3	15	.31	15	.26	30	.37	29	.08
1954	2	32	.29	32	.30	1024	.36	22	.06
1955	2	32	.37	32	.40	1024	.48	24	.09

Heterosis is obtained by comparing the cross to the mid-parent. The average value of heterosis, giving equal weight to each year's estimate is 26 percent of the mid-parent mean or 0.08 pounds per plant.

The estimates of σ^2_m and σ^2_f made in each Design I test of the parental varieties and the variety cross are presented in Table 3.

All of the results with the varieties and their cross were obtained from unselected varietal parent material. Each of two samples of Jarvis and one sample of Indian Chief intravariety progenies were tested at the same location for two years. Each of two samples of the variety cross populations were tested at two locations in a single year. Whereas the samples of Jarvis and the variety cross populations were of 64 male groups or 256 progenies, a double sample of Indian Chief consisting of 128 male groups or 512 biparental progenies was tested. In order that the results be comparable with respect to the expected contribution of genotype environmental interactions for all entries, the estimates were made for each year and location (Table 2).

As can be seen from Table 3 the estimates of components of variance within varieties are generally larger than those within the cross. Comparisons of the averages for the varieties and crosses are made in Table 4, where the differences and ratios are also computed. The genetic variances were enough higher in the varieties than in the crosses to give ratios of 1.96 and 1.52 (Table 4).

Theoretical

The procedure to be followed will be to examine the intra- and intervariety results as to their compatibility with the simple genetic model of only additive and dominance effects of genes and two alleles per locus. It will be further assumed that there are no maternal or cytoplasmic effects and that the varieties are in Hardy-Weinberg equilibrium as well as in linkage equilibrium.

The following symbols will be used to generate the needed expressions:

p = relative frequency of the favorable gene in one variety (V_1)

q = relative frequency of the same gene in the other variety (V_2)

TABLE 3

Estimates of σ_m^2 and σ_f^2 for Jarvis, Indian Chief and their reciprocal F_1 crosses

Population	Year	Location*	σ_m^2	σ_f^2
Jarvis	1950	1	.00098	.00150
	1951	1	.00146	.00195
	1952	1	.00067	.00040
	1953	1	.00133	.00137
	Avg.		.00111	.00130
Indian Chief	1952	1	.00077	.00071
	1953	1	.00083	.00173
	Avg.		.00080	.00122
Means for varieties			.00096	.00126
	1954	1	.00035	.00173
Jarvis	1954	2	.00044	.00108
×	1955	1	.00032	.00082
Indian Chief	1955	2	.00064	.00037
	Avg.		.00044	.00100
	1954	1	.00043	.00079
Indian Chief	1954	2	.00055	.00081
×	1955	1	.00049	.00012
Jarvis	1955	2	.00071	.00091
	Avg.		.00054	.00066
Means for crosses			.00049	.00083

* Central Crops Research Station, Clayton = 1
Upper Coastal Plain Research Station, Rocky Mount = 2

TABLE 4

Comparison of intra- and intervarietal variance estimates

Comparison	Components	Estimates	Difference	Ratio
1	$(\sigma^2_{m_{11}} + \sigma^2_{m_{22}})/2$	.00096 ± .00008*		
			.00047 ± .00011	1.96
	$(\sigma^2_{m_{12}} + \sigma^2_{m_{21}})/2$	.00049 ± .00008		
2	$(\sigma^2_{f_{11}} + \sigma^2_{f_{22}})/2$	.00126 ± .00020		
			.00043 ± .00028	1.52
	$(\sigma^2_{f_{12}} + \sigma^2_{f_{21}})/2$	.00083 ± .00020		

* These standard errors were arrived at by estimating variances among the estimates of σ_m^2 and of σ_f^2 within varieties or crosses in Table 3 and then pooling over varieties and crosses.

$2u$ = the mean phenotype of the superior homozygote minus the mean phenotype of the inferior homozygote.

au = the mean phenotype of the heterozygote minus the mean phenotype of the average of the two homozygotes.

a describes dominance in the following way: $a < 0$ is negative dominance, $a = 0$ is no dominance, $0 < a < 1$ is partial dominance, $a = 1$ is complete dominance and $1 < a$ is overdominance.

Using procedures followed by COMSTOCK and ROBINSON (1948) the following expressions are found:

(a) Heterosis measured as the variety cross mean minus the average of the two varieties:

$$\sum (p - q)^2 au \tag{1}$$

Where the summation is over all loci.

(b) Intravariety male component of variance (example for V_1):

$$\sigma^2_{m_{11}} = \tfrac{1}{2} \sum p(1-p)[1 + (1-2p)a]^2 u^2. \tag{2}$$

Within a variety, $4\sigma^2_{m_{11}} = \sigma^2_{g_{11}}$, the additive genetic variance. An analogous expression of $\sigma^2_{m_{22}}$ for V_2 is obtained by substituting q for p.

(c) Intravariety component of variance for females mated to the same male (example for V_1):

$$\sigma^2_{f_{11}} = \tfrac{1}{2} \sum p(1-p)[1 + 2(1-2p)a + (1 - 2p + 2p^2)a^2]u^2 \tag{3}$$

$$= \sigma^2_m + \sum p^2(1-p)^2 a^2 u^2. \tag{4}$$

Within a variety, $4(\sigma^2_{f_{11}} - \sigma^2_{m_{11}}) = \sigma^2_{d_{11}}$, the dominance variance.

(d) Intervariety male component of variance (example for V_1 males crossed onto V_2 females):

$$\sigma^2_{m_{12}} = \tfrac{1}{2} \sum p(1-p)[1 + (1-2q)a]^2 u^2. \tag{5}$$

The male component, $\sigma^2_{m_{21}}$, for the reciprocal cross is obtained by interchanging p and q.

(e) Intervariety female component of variance (example for V_1 males crossed onto V_2 females):

$$\sigma^2_{f_{12}} = \tfrac{1}{2} \sum q(1-q)[1 + 2(1-2p)a + (1 - 2p + 2p^2)a^2]u^2 \tag{6}$$

$$= \sigma^2_{m_{21}} + \sum q(1-q)p(1-p)a^2 u^2 \tag{7}$$

The difference between the intravariety male components and the intervariety male components is

$$\tfrac{1}{2}(\sigma^2_{m_{11}} + \sigma^2_{m_{22}}) - \tfrac{1}{2}(\sigma^2_{m_{12}} + \sigma^2_{m_{21}})$$

$$= \sum (p-q)^2(p+q-1)[1 - (p+q-1)a]au^2. \tag{8}$$

In addition to the condition that $p \neq q$ or $(1 - q)$, the two sets of admissible conditions for which a locus' contribution to this difference will be positive are:

1. $$0 < a, 1.0 < p + q < 1 + \tfrac{1}{a} \tag{9}$$

and

2. $$a < 0, 1 + \tfrac{1}{a} < (p + q) < 1. \tag{10}$$

For future reference it is pointed out that the conditions for which the difference (8) is positive are those for which the ratio

$$(\sigma^2_{m_{11}} + \sigma^2_{m_{22}})/(\sigma^2_{m_{12}} + \sigma^2_{m_{21}}) \tag{11}$$

is greater than one.

The difference between the intra- and intervariety female components of variance is

$$\frac{1}{2}(\sigma^2_{f_{11}} + \sigma^2_{f_{22}}) - \frac{1}{2}(\sigma^2_{f_{12}} + \sigma^2_{f_{21}})$$

$$= \frac{1}{2}\Sigma (p-q)^2 (p+q-1)\,[2-(p+q-1)a]au^2, \tag{12}$$

and the sets of conditions in addition to the one that $p \neq q$ or $(1-q)$ for a locus to make a positive contribution to this difference are:

1. $$0 < a,\ 1 < p+q < 1+\frac{2}{a} \tag{13}$$

and

2. $$a < 0,\ 1+\frac{2}{a} < p+q < 1 \tag{14}$$

By reference to (2), (4), (5) and (7) it will be noted that the difference expressed in (12) contains the difference expressed in (8) plus the difference between fractions of the intravariety dominance variances and analogous intervariety variances. This latter difference is

$$\frac{1}{2}[(\sigma^2_{f_{11}} - \sigma^2_{m_{11}}) + (\sigma^2_{f_{22}} - \sigma^2_{m_{22}})] - \frac{1}{2}[(\sigma^2_{f_{12}} - \sigma^2_{m_{21}}) + (\sigma^2_{f_{21}} - \sigma^2_{m_{12}})]$$

$$= \frac{1}{2}\Sigma (p-q)^2 (p+q-1)^2 a^2 u^2, \tag{15}$$

which is never negative. This accounts for the conditions being less stringent for positiveness of (12) than of (8).

Since the conditions are less stringent for (12) than for (8) to be positive it will be necessary to investigate the variations in conditions for only (8). Table 5 gives the ratios (equation (11)) for a single locus with various selected values of p, q and a. Positive values of a equal to or less than 1.0 are considered for the gene frequencies $(p+q > 1.0)$ that give rise to ratios of the variances that equal to or exceed 1.00. The value of a must be positive for most loci for yield in corn since heterosis is generally positive (see equation (1)). Values of a greater than one are not considered, primarily, because ROBINSON *et al.* (1955) concluded from the smallness of $(\sigma^2_{f_{11}} - \sigma^2_{m_{11}})/\sigma^2_{m_{11}}$ and $(\sigma^2_{f_{22}} - \sigma^2_{m_{22}})/\sigma^2_{m_{22}}$ that dominance was partial to complete for the majority of the loci for yield. Also, many more combinations of p and q will give a ratio greater than one when dominance is partial to complete than when there is overdominance.

The ratios for any combination of p and q in Table 5 increase with an increase in a. Holding the average of p and q constant, the ratios increase with an increase in the difference between p and q and holding the difference between p and q constant, the ratios increase with an increase in the average of p and q. Ratios

greater than 1.5 are found for small differences in gene frequencies when gene frequencies are high and dominance is close to complete.

By reference to Table 5 it is seen that the ratios found in Table 4 are very plausible for the model investigated here. Many conditions given in terms of com-

TABLE 5

Ratios of $(\sigma^2_{m_{11}} + \sigma^2_{m_{22}})/(\sigma^2_{m_{12}} + \sigma^2_{m_{21}})$ *for selected values of* p, q *and* a

		a			
p	*q*	.25	.50	.75	1
.60	.50	1.00	1.00	1.00	1.00
.70	.40	1.02	1.04	1.06	1.08
	.50	1.02	1.04	1.06	1.09
	.60	1.01	1.02	1.03	1.04
.80	.30	1.07	1.14	1.21	1.26
	.40	1.09	1.19	1.29	1.38
	.50	1.07	1.16	1.27	1.38
	.60	1.05	1.10	1.18	1.27
	.70	1.02	1.04	1.07	1.11
.90	.20	1.22	1.44	1.63	1.74
	.30	1.28	1.63	1.99	2.25
	.40	1.27	1.65	2.11	2.51
	.50	1.23	1.56	2.03	2.54
	.60	1.17	1.42	1.82	2.34
	.70	1.10	1.25	1.51	1.94
	.80	1.03	1.09	1.19	1.40
.95	.10	1.29	1.59	1.80	1.89
	.20	1.50	2.21	2.93	3.33
	.30	1.53	2.40	3.53	4.33
	.40	1.48	2.34	3.69	4.89
	.50	1.40	2.15	3.50	5.01
	.60	1.30	1.88	3.03	4.70
	.70	1.20	1.58	2.41	3.96
	.80	1.10	1.29	1.73	2.84
	.90	1.02	1.06	1.15	1.46
.99	.05	1.81	3.08	4.33	4.79
	.10	2.04	4.18	7.36	9.08
	.20	2.06	4.68	10.67	16.13
	.30	1.95	4.43	11.51	21.11
	.40	1.81	3.94	10.86	24.00
	.50	1.66	3.37	9.35	25.00
	.60	1.51	2.80	7.44	24.00
	.70	1.36	2.25	5.45	21.00
	.80	1.22	1.74	3.56	16.00
	.90	1.09	1.30	1.96	9.00
	.95	1.03	1.10	1.31	5.00

binations of p, q and a, give ratios comparable to those estimated. While the observed ratios are of components which are summed over all loci that can vary in gene frequencies, gene effects and dominance, the theoretical ratios in Table 5 are computed for a single locus. The observed ratios represent average values while the theoretical ones are specific. At least part of the segregating loci for yield must exhibit individual ratios as great or greater than the average. For a single locus and for the conditions of (9) under consideration, the intervariety components must lie between the two intravariety components. However, one variety will have the largest component for part of the loci and the other variety will have the largest component for the remaining loci, depending upon the gene frequencies in the two varieties. Thus the results can well be that the estimable components, which are summed over all loci, for the varieties are each larger than the corresponding components for the crosses as observed in Table 3.

DISCUSSION

The most widely accepted hypothesis for explanation of heterosis remains the "dominance of favorable genes" proposed by BRUCE (1910). While objections have been raised to this hypothesis as the complete explanation, and properly so, it is still the simplest and most acceptable argument. The question of level of dominance has attracted considerable attention, due largely to the proposal by HULL (1945), CROW (1948) and others that overdominance may be important.

The observed genetic variances within varieties and their cross together with the observed heterosis appear probable on the basis of additive model with dominance. Partial to complete dominance of the genes with high, but different gene frequencies in the two varieties, is a suggested genetic situation in the population that is in agreement with the total experimental findings.

There is little doubt that there are gene frequency differences between these varieties. Their general agronomic appearance, history of maintenance and perpetuation, performance of inbred lines that have been extracted from each, in addition to the heterosis observed when crossed, require that they differ in gene frequencies. However, the extent of their gene frequency differences with respect to yield is not known, and with the exception of the heterosis, other observed differences may bear little relationship to gene differences for yield. The yield of the parental varieties is approximately equal as shown in the result of Table 2 where the average yield of Jarvis is only slightly greater than that of Indian Chief.

The variety differences measured in this study are not a result of the varieties being fixed for different alleles. Inbreeding or drift of the varieties could well account for differences in gene frequencies between the varieties, but not to the extent that they have drifted to fixation because fixed loci would not contribute to differences between the variances of varieties and their crosses. This does not rule out the possibility, suggested by ROBINSON *et al.* (1957), that the varieties are homozygous for different alleles at many loci. Variation from these genes can be measured only in later segregating generations of the cross.

It was realized from the outset that it is a bit hazardous to compare genetic variances of different populations where estimates were obtained under different environmental conditions. All components were computed so that they contained interactions of genotypes with years, locations, and years by location. Should they contribute to the male or female component of variance in proportion to the genetic variance that the component contains, then the ratios (Table 4) are unaffected by these interactions. If, on the other hand, the genotype environment contributions to all components in the ratio are the same, then the differences and the ratios in Table 4 are less than they should be, and no conclusions are altered. However, the components for the parents were estimated in different years from those for the cross. Thus, year differences are confounded with the comparison of crosses and varieties. While complete validity of the comparison can in no way be insured, the consistency of estimates reported in Table 3 lends credence to their value. Variance among the estimates in Table 3 are among estimates for different years and/or locations. Using this variance as an error term, the male component of variance in the varieties is significantly greater than in the crosses. Also, the results for the second location in the crosses agrees fairly well with those for the first location.

It is believed to be a general truth that a more general genetic model can explain results which are compatible with a simpler model. Since these results agree with the simple additive model with dominance, they, therefore, offer no evidence against or for multiple alleles, epistasis and linkages. Linkages would affect the results only if the varieties were out of linkage equilibrium. Until there is evidence to the contrary, there is merit in holding to the simplest explanation; in this case, the additive model with partial to complete dominance.

SUMMARY

This study consisted of a joint consideration of observations on genetics variances in two open pollinated varieties of corn and in the cross of these varieties and on the heterosis exhibited by the variety cross.

Interpretation of the results for yield related to:

1. Heterosis in the varieties cross exceeded the mid parent value by an amount greater than 20 percent.
2. The component of variance due to genetic differences between male parents was greater within the varieties than in the variety cross.
3. The component of variance due to genetic differences between female parents mated to the same male was likewise greater in varieties than in the variety cross.

The results were in agreement with the model assuming additive gene effects with partial to complete dominance and no epistasis or multiple alleles.

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DEVELOPMENTAL GENETICS OF THE POSTERIOR LEGS IN DROSOPHILA MELANOGASTER

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ONE of the characteristic morphological features of certain of the species of the Drosophiladae is the sex comb (ctenidium) on the first and in some species also the second tarsal joint of the prothoracic leg of the male but never the female. The males of *Drosophila melanogaster* have a single comb of 10–12 morphologically distinct bristles (teeth) on the distal end of the first tarsal segment, but occasionally a single tooth or several teeth occur as a developmental anomaly on the other tarsal segments of the first leg or on the other legs.

Five mutants are known in *D. melanogaster* which induce the formation of typical sex combs on the metatarsi of the posterior legs. If sex comb teeth are developed on the metatarsi of the posterior legs, it may be because the genetic factor causes differentiation of a sex comb only, and this quite independent of differentiation in the rest of the tarsus or the rest of the leg. Conversely an extra sex comb mutant may have a more profound morphogenetic effect. It may redirect the development pattern so that the posterior legs become first legs with the appropriate region of the male metatarsus differentiating a sex comb.

The legs of *D. melanogaster* are admirably suited for morphogenetic studies for the three legs are differentiated on the basis of size, structure and chaetotaxy (HANNAH-ALAVA, 1958). In addition the metatarsus of the first leg is sexually dimorphic in the total chaetotaxal pattern as well as gross anatomy and certain easily distinguishable bristle groups. A preliminary study by HANNAH (STERN 1954b) showed that some of the extra sex comb mutants did indeed induce profound changes in the metatarsi of the posterior legs of which the sexcombs were only the most obvious morphological change. A detailed analysis, presented in this paper, substantiates the hypotheses that the extra sex comb mutants do not induce sex combs *per se*, but that they are primarily concerned with changing the prepattern in the posterior legs, and after the prepattern is changed development of the legs continues in an orderly fashion but in a new direction. The results also show that the changes in the posterior legs of the female are just as profound as in the male, but always in the direction of the female first leg, as the changes in the posterior legs of the male are in the direction of a male first leg.

MATERIALS AND METHODS

Morphogenetic studies were made of a hybrid between two wild type strains and various combinations of three extra sex comb mutants: extra sex comb

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(*esc*), Polycomb (*Pc*) and Extra sex comb (*Scx*). Consequently it was possible to compare the three mutants not only in simplex but their interactions in compounds.

Genotypes

Wild type: For comparison with the extra sex comb genotypes, offspring from Samarkand females (from a strain inbred 269 generations) and Oregon-R males (from a strain inbred 171 generations) were used as the normal wild type. The Samarkand-Oregon hybrid will be referred to as Sam-Ore or wild type in the text.

extra sex comb: The first extra sex comb mutant, *esc*, found and described by SLIFER (1942) is a second chromosome recessive factor characterized by sex combs on each of the six legs of the male. It is homozygous sterile in both sexes. SLIFER noted especially the variable penetrance, but also reported that it could be increased by a liberal diet of fresh yeast. More recent studies (HANNAH and STRÖMNAES 1955, HANNAH-ALAVA, unpublished) have shown that in normal cultural conditions at least one extra sex comb is present in the majority of esc males, and that both penetrance and expressivity are affected by different cultural media and particularly by crowding during development.

Polycomb: Pc, a third chromosome dominant mutant (lethal in homozygotes) was found and described by P. LEWIS (1947). It is also characterized by sex combs on all three pairs of legs in the male as well as by other phenotypic effects such a elevated, divergent or crinkled wings and bent humeral and anterior notopleural bristles (LEWIS 1947, HANNAH and STRÖMNAES 1955). As a heterozygote with wild type (*Pc*/+), it has a much lower penetrance and expressivity than homozygous *esc*. Under normal conditions from 50–75 percent of the males have a comb (seldom of more than four teeth) on one or both of the second legs and very rarely even a single tooth on the third legs. With poor cultural condition, crowding or on certain media the extra sex comb characters may not be expressed at all.

Extra sex comb: Scx is also a third chromosome dominant and like *Pc* is lethal in homozygotes. As a heterozygote, (*Scx*/+), penetrance and expressivity is between Pc and esc (HANNAH and STRÖMNAES 1955). Like Pc it is very sensitive to environmental conditions, but the third leg is more sensitive than the second. In normal conditions penetrance is between 75–90 percent with both legs being affected in over half of the flies; in a less favorable environment the penetrance drops to below 50 percent with the third legs rarely having sex combs. The pleiotropic effects on other parts of the body, characteristic of Pc, are less extreme or do not occur in Scx flies.

The *Scx*/*Pc* compound is neither lethal nor sterile, but the flies have somewhat lower viability than either *Pc*/+ or *Scx*/+ flies. The sex comb character is completely penetrant except in very poor environmental conditions and most of the males have "good" sex combs on all six legs (HANNAH and STRÖMNAES 1955). In addition to the leg effects, the other pleiotropic effects characteristic of Pc are the same or even accentuated in *Scx*/*Pc* flies; the sternopleural bristles are frequently missing, the humerus is depressed, and the wings are spread or drooping.

The *esc/esc; Scx/+* compound is completely penetrant for the extra sex comb characters and has almost normal viability. The other pleiotropic effects, characteristic of Pc and Pc compounds, are very slight, if present at all.

The *esc/esc; Scx/Pc* compound is almost lethal, particularly in the female. The surviving flies are smaller in size, weak, highly sterile, and frequently have legs so abnormal that an analysis of the chaetotaxy is impossible. All of the pleiotropic effects are very extreme and of the type described for *Pc/+* and *Scx/Pc* flies.

The extra sex comb strains were maintained as heterozygotes: *esc/Bl*, *Pc*/T-(2;3)*Mé*, and *Scx/In(3)* T M, *Mé* (for a description of the markers see LEWIS and GRELL 1953; BRIDGES and BREHME 1944). Compounds were made in several ways: *Pc/+* and *Scx/+* flies were obtained from matings of Samarkand females and *Pc/Mé* or *Scx/Mé* males; *esc/esc* flies from crosses of *esc/Bl* females and males; *Scx/Pc* from *Pc/Mé* × *Scx/Mé* matings, *esc/esc; Scx/+* flies from crosses of *esc/Bl*×*Cy/Pm; Scx Sb/+* and the F_1 *esc/Cy; Scx Sb/+* males backcrossed to *esc/Bl* females; and the *esc/esc; Scx Sb/Pc* flies from crosses of *esc/Bl; Scx Sb/Pc* females and males which had been synthesized by appropriate crosses. (Comparison of *Scx Sb/+* to *Scx/+* flies showed that *Sb* had little or no effect upon the expression of the extra sex comb phenotype. This is in agreement with DOBZHANSKY's (1930) observation that Sb flies did not differ from wild type flies in size or shape of the legs, and with HANNAH-ALAVA and STERN's (1957) finding that *Sb* did not affect either the size or number of sex comb teeth. Consequently *Sb* will not be included in subsequent designations of the genotypes having this marker.)

A simplified form of the genotypes will be used throughout this paper: esc = *esc/esc*, Scx = *Scx/+*, Pc = *Pc/+*, esc-Scx = *esc/esc; Scx/+*, Scx-Pc = *Scx/Pc* and esc-Scx-Pc = *esc/esc; Scx/Pc*.

Techniques

Method of culture: The standard culture medium was employed: cornmeal, agar, molasses and brewer's yeast with the addition of live yeast just before using the culture bottle. Four to eight pairs of flies, of the appropriate genotypes, were permitted to lay eggs for about four days in one bottle then transferred to a fresh bottle for about four to five days before discarding. All cultures were incubated at 26 ± 1° C. Offspring were checked within one day after emergence, then either preserved in alcohol or mounted.

Mounting and recording: In all genotypes, except esc-Scx-Pc in which only 16 males and four females were available for mounting, at least 30 mounts were made of each sex for each genotype. To include a representative sample of the various phenotypes larger samples were made for the genotypes with a low penetrance of the sex comb character. The three pairs of legs were cut from the body of the fly as a unit (or if the pairs were separated they were placed in the same relative position) and mounted in euparol. More recently a technique described by STRÖMNAES and HANNAH (1955) which facilitated mounting was used, after confirmatory tests to show that legs so treated were not distorted in size or shape.

Every leg which was in a position suitable for a chaetotaxal study was recorded. The legs were studied with a compound binocular microscope at magnifications ranging from 100–600 ×. The length of each tarsus was measured on an arbitrary scale (using a mm rule) from a camera lucida drawing. At the magnification used for such measurements 1 unit = 3.8μ.

In addition to the general morphological observations, more detailed studies were made of the number and position of the sex comb teeth, the number and position of the chaetae in the distal part of the tibia, all of the metatarsus (first tarsus) and selected portions of the femur and coxa; in other words a study of the chaetotaxal pattern. In describing the position of the bristles a modification of GRIMSHAW's system (1905) was used. Thus the surfaces of the segments are described as if the leg were stretched horizontally from the body, with the outer surface (and the preapical bristle) being dorsal and the inner surface (and the apical bristle) being ventral (see HANNAH-ALAVA 1958 for details). To facilitate recording, the chaetotaxal pattern was charted for each leg in a table and upon diagrams similar to those given in Figure 1. In addition camera lucida drawings were made of many of the legs, and of these representative ones were selected for the figures in this paper.

OBSERVATIONS

Morphology and chaetotaxy of the legs of Sam-Ore flies

A detailed morphological analysis of the legs of wild type flies (HANNAH-ALAVA, 1958) showed that there are extensive differences between the three legs in size, shape and chaetotaxy, and that the chaetotaxal pattern, among the different normal genotypes is relatively stable. Secondly, the chaetotaxy of the metatarsus of the first leg is sexually dimorphic both in respect to the pattern and the number of bristles per row in the proximal-distal rows. The chaetotaxal patterns in the second and third legs are not sexually dimorphic but the female consistently has a higher mean number of bristles in each of the proximal distal rows. The number of bristles per row in either sex was not covariant with the tarsus length, thus the variation in bristle number is not proportional to, or dependent upon tarsal length. Thirdly, although the mean number of bristles per row in rows 1, 3–5 and 8 in the metatarsi of the three legs is not the same, because of the position of the rows and the presence of the small bractless bristles in the same relative position between the appropriate rows, there is little doubt that these rows are equivalent and probably homologous.

Diagrams of the typical chaetotaxal pattern of the metatarsus and the distal end of the tibia for the three legs of Sam-Ore males are given in Figure 1, A, C and D and the female first leg in Figure 1B. These diagrams show the 13 "characters" studied: the proximal-distal rows 1 to 8; row 5.5, a group of bractless bristles between rows 5 and 6; the tarsal transverse rows; the tibial transverse rows; the number of sex comb teeth; and the length of the metatarsus. The mean value for each character for the legs of the male is given in Tables 1 and 4 and of the female in Tables 7 and 8.

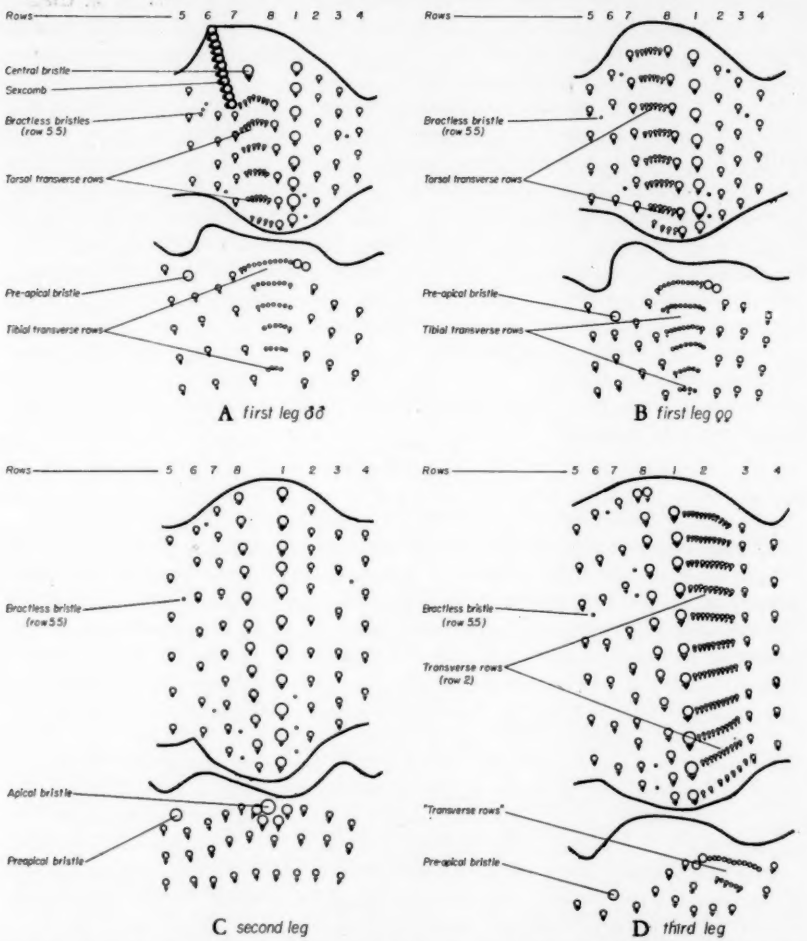

FIGURE 1.—Typical chaetotaxal pattern of the legs of Sam-Ore hybrids (For details see HANNAH-ALAVA 1958). The numbered rows (above each figure) indicate the proximal-distal rows on the tarsus. The circles indicate bristles and the triangles below some of the circles indicate that these bristles have associated bracts. The other characters are labeled in each figure. A.—Diagram of the chaetotaxy of the metatarsus and distal end of the tibia of the first leg of a male. B.—Same for the first leg of a female. C.—Same for the second leg of a male. D.—Same for the third leg of a male.

Effect of extra sex comb mutants upon differentiation of the meso- and metathoracic legs

On the assumption that the second and third legs are transformed by the extra sex comb mutants into first legs, the following morphological or chaetotaxal changes would be expected to occur in the metatarsi of flies with one or more extra sex comb mutants: a decrease in the length of the tarsus accompanied by a decrease in the number of bristles per row in rows 1 to 8; an increase in the male of the number of bractless bristles between rows 5 and 6; formation of transverse rows of bristles between rows 7 and 8 in both male and female legs; formation of a sex comb and a central bristle in the male tarsus. In the tibia the changes expected are: formation of the transverse rows at the distal end; and reduction in the size of the apical bristle of the second leg and its incorporation into the most distal transverse row of bristles. Changes in the more proximal segments would be: development of a complete row of larger bristles along the length of the posterior surface and several preapical bristles on the anteriodorsal surface of the femur; and changes in the shape, size and chaeotaxy of the coxa.

The evidence, to be presented, shows that all of these changes occur and that they occur increasingly with an increasing number of extra sex comb factors.

A. Changes in the metatarsus and tibia of the male legs

Mesothoracic legs: Comparisons of the chaeotaxal pattern and length of the mesothoracic metatarsus of Sam-Ore males with males having various combinations of the extra sex comb factors are presented in Table 1, and representative types in Figure 2. The mean number of teeth in the sex comb increased from none in Sam-Ore males to 12.3 in esc-Scx-Pc flies. The tarsal and tibial transverse rows, which are not present in the wild type males, occurred in flies with one extra sex comb factor and increased in number with an increase in number of factors. Thus, all of the chaetotaxal characters normally present only in the first leg occur in the second leg of flies with one or more extra sex comb factors. Concurrently with the induction of the first-leg characters, there was a decrease in tarsal length and a decrease in the number of bristles per row in all eight proximal-distal rows. Although individually the mutants do not cause a complete transformation of the second leg into a first leg, in the esc-Scx-Pc compound the second legs have virtually become first legs for all 13 characters (Table 1). These results are in support of the hypothesis that the primary function of an extra sex comb mutant is to transform a posterior leg into a first leg, not just to induce the formation of a sex comb. The data also suggest that there is a systematic variance for all of the characters in a single genotype or among the genotypes.

The mean expression for each genotype cannot be obtained directly from the means of the characters for they are based upon diverse criteria. In order to evaluate them, each genotype was ranked from one to seven— among the genotypes—for each of the 13 characters. (In ranking, the standard error was taken into consideration; it was assumed that means that overlapped up to 2 S.E. were

TABLE 1

Comparison of the chaetotaxal pattern in the first leg of Sam-Ore males with the pattern in the second legs of males from Sam-Ore and the six extra sex comb genotypes

Genotype Sample	Sam-Ore 20/50 1st leg	Sam-Ore 20/50 2nd leg	*Pc*/+ 35/104	*Scx*/+ 88	*esc/esc* 20/81	*Scx/Pc* 43	*esc/esc; Scx*/+ 20/54	*esc/esc; Scx/Pc* 20/32
Row 1*	7.8±0.1	11.2±0.2	11.9±0.1	11.3±0.1	11.3±0.2	9.7±0.2	9.0±0.2	7.8±0.1
Row 2	6.3±0.1	10.0±0.1	10.1±0.1	9.7±0.1	9.6±0.2	7.6±0.2	7.7±0.2	6.5±0.2
Row 3	4.7±0.1	6.6±0.2	7.1±0.1	6.7±0.1	6.9±0.1	5.8±0.1	5.9±0.1	5.6±0.1
Row 4	4.0±0.1	5.8±0.1	6.0±0.1	5.5±0.1	5.8±0.1	4.7±0.1	4.8±0.1	4.2±0.1
Row 5	4.2±0.1	6.1±0.1	6.2±0.1	5.6±0.1	5.7±0.2	3.9±0.1	4.6±0.2	3.8±0.1
Row 5.5	3.7±0.1	1.0±0.0	1.7±0.1	2.5±0.1	2.1±0.2	4.0±0.1	3.9±0.2	4.4±0.2
Row 6	3.9±0.1	7.0±0.2	6.3±0.1	5.8±0.1	6.0±0.1	3.6±0.1	3.7±0.1	3.3±0.1
Row 7	5.2±0.2	9.5±0.2	9.1±0.2	7.9±0.2	8.4±0.2	5.2±0.1	5.1±0.1	5.0±0.2
Row 8	6.4±0.1	11.9±0.2	12.1±0.1	11.3±0.2	11.1±0.3	7.0±0.2	6.5±0.2	6.2±0.1
Tarsus length	47.8±0.2	76.7±0.3	76.3±0.2	66.9±0.3	68.0±0.4	53.7±0.4	51.6±0.5	44.7±0.5
Sex comb teeth	11.0±0.1	....	0.6±0.1	4.7±0.4	5.2±0.3	11.2±0.2	11.3±0.2	12.3±0.2
Ta. tr. rows†	5.8±0.1	....	0.0±0.0+	1.4±0.2	1.1±0.1	6.2±0.2	5.8±0.1	5.9±0.1
Ti. tr. rows‡	6.1±0.1	....	0.1±0.1	1.6±0.2	1.9±0.2	5.3±0.1	5.6±0.1	5.5±0.1

The entries in each column include the mean±1 S.E. for 13 characters: the number of bristles per row in each of the proximal-distal rows, the number of sex comb teeth, the number of tibial and tarsal transverse rows and the length of the tarsus in units (1 unit=3.8μ). The mean (and standard error) is based upon a sample of 20 legs for the rows and a sample of 50 legs for the length, sex comb teeth and transverse rows in Sam-Ore, 35 legs and 104 legs respectively for *Pc*, etc.; *Scx* and *Scx-Pc* were completely sampled, 88 legs and 43 legs respectively.

* Row–1—Row–8. Proximal-distal rows of the tarsus.
† Tarsal transverse rows.
‡ Tibial transverse rows.

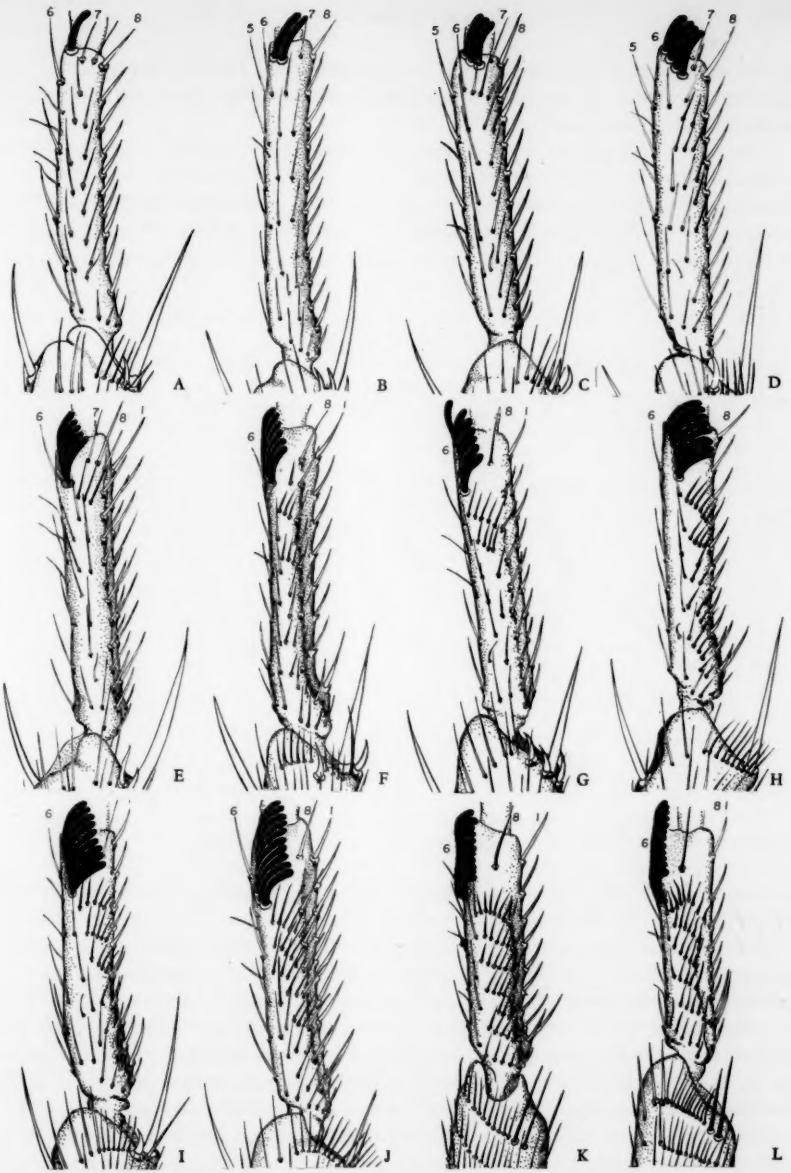

FIGURE 2.—Stages in the transformation of a male second leg into a first leg through changes in size of the tarsus and chaetotaxy of the tarsus and tibia. A-L—Examples of the metatarsus and distal end of the tibia from second legs of males with one or more extra sex comb factors (A-J from Scx and K-L from Scx-Pc). The newly induced or transformed bristles of the transverse rows are in solid black; the bristles characteristic of the second leg are in outline. For details see Figures 1A and C.

of the same rank.) The mean rank for each genotype was derived by averaging the 13 rank values. On the basis of the mean rank, the genotypes form a graduated series in the following order:

Genotype:	Sam-Ore	>	Pc	>	esc	>	Scx	>	Scx-Pc	>	esc-Scx	>	esc-Scx-Pc
Mean rank:	1.69		2.09		2.88		3.35		5.65		5.77		6.58

Hence according to their effects, the genotypes can be arranged in a linear series, and a series correlated with the increase in number of extra sex comb factors in the genotypes (Figure 3). The mean rank values also show that the genotypes

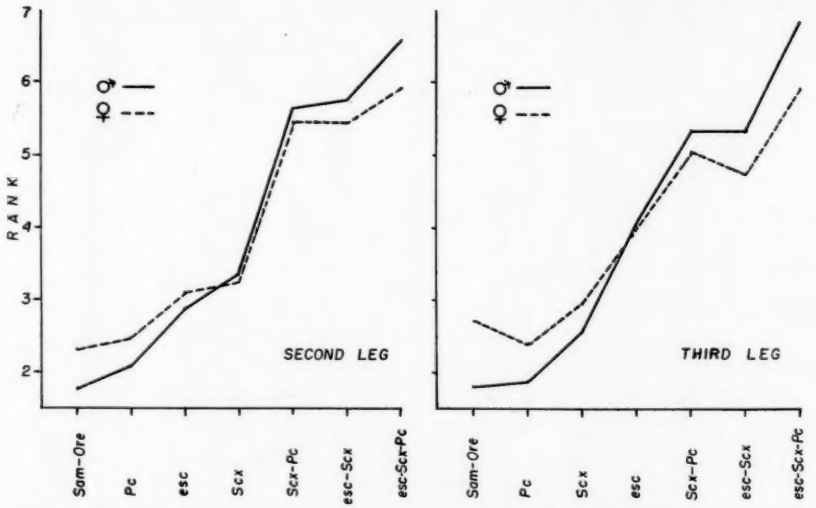

FIGURE 3.—Comparison of the mean rank for each genotype after ranking the seven genotypes for each of the 13 characters in the male and the 12 characters in the female.

overlap in expression of the characters: Sam-Ore has a mean rank of 1.69 instead of 1.00 and esc-Scx-Pc 6.58 instead of 7.00.

These results also point to the probability that the expression of the various characters is correlated. This was tested in two ways: (1) the flies of a specific genotype were divided into classes on the basis of one of the characters (such as number of sex comb teeth) and the means of the other characters tabulated as they were for the genotypes in Table 1; and (2) any two characters were evaluated by means of a correlation test. The first method permits a comparison of all of the characters simultaneously, but only the means without taking into consideration the variances. The second method permits comparison of variance between two characters only but has the advantage that it is simultaneously a measure of covariance.

(1) *Systematic variation within a genotype:* A complete chaetotaxal study of a large sample of flies was made for only two genotypes, Scx and Scx-Pc. Of the

Scx flies, only four had no sex combs on either second leg, the remaining 40 flies had from none to 11 teeth per comb. In the Scx-Pc flies the number of teeth per comb ranged from 8 to 13. As this is the most conspicuous character the legs were separated into classes on the basis of the number of sex comb teeth per leg.

In the five classes of Scx (that is flies with none, 0–2, 3–5, 6–8, and 9–11 teeth per leg) the other characters, with minor exceptions, showed the expected increase or decrease consistent with the increase in number of sex comb teeth (Table 2).

TABLE 2

Evidence for a systematic change in the expression of 13 characters in the second leg and in one- and two-factor genotypes

Genotype	*Scx/+*					*Scx/Pc*	
No. of teeth	0	0–2	3–5	6–8	9–11	8–11	12–13
Sample	8	19	25	17	19	24	19
Row 1	12.0	11.3	11.3	11.1	10.9	9.7	9.8
Row 2	10.5	9.8	9.7	9.7	9.3	7.5	7.8
Row 3	7.0	6.9	6.7	6.7	6.4	5.9	5.6
Row 4	5.5	5.4	5.4	5.2	5.5	4.7	4.7
Row 5	6.0	5.7	5.5	5.4	5.6	3.8	3.9
Row 5.5	1.0	1.6	2.4	2.8	3.8	3.9	4.1
Row 6	6.9	7.0	5.8	5.4	4.6	3.3	3.8
Row 7	9.6	9.4	7.8	7.1	6.4	5.2	5.1
Row 8	13.3	11.9	11.4	11.1	9.8	7.1	6.9
Tarsus length	69.6	69.5	66.6	65.8	64.5	53.2	54.3
Sex comb teeth	0.0	0.8	3.9	7.1	9.7	10.5	12.2
Ta. tr. rows	0.0	0.1	1.2	2.2	3.2	6.0	6.4
Ti. tr. rows	0.0	0.3	1.7	2.0	3.1	5.2	5.4

The 88 legs from *Scx* males were divided into five groups: flies with no sex comb teeth on either leg, and (of the flies having at least one comb on a posterior leg) second legs with 0–2, 3–5, 6–8 and 9–11 teeth per comb. The 43 legs of *Scx-Pc* males were separated into two groups, those having 8–11 and those having 12–13 teeth per comb in the second leg. Abbreviations are the same as in Table 1.

Thus the progressive seriation for the 13 characters within the Scx genotype completely paralleled the progressive seriation found among the genotypes. It seems probable that in the Scx genotype a single factor—the extra sex comb factor—is inducing all of the changes and that modifying factors (unless they affect the characters as a unit) are not of significance.

In the two factor compound, Scx-Pc, the flies were divided into two classes, those with 8–11 and those with 12–13 sex comb teeth. The differences between the classes is not great, in fact in some of the characters the legs with the larger number of sex comb teeth actually deviate in the opposite direction from that expected, that is more bristles per row instead of fewer, longer length of the metatarsus instead of shorter. Consequently, in Scx-Pc the seriation is not evident. This probably does not mean that the expressions of the characters are not correlated in this genotype but only that the variance may be great enough to mask a systematic difference.

(2) *Covariance:* Sufficient data were not available for complete correlation tests for Pc. Of the 104 second legs studied only 43 expressed the extra sex comb pheno-

type: four had only tibial or tarsal rows, 27 had only a sex comb (of one to four teeth), and 12 had a sex comb and tibial and/or tarsal rows. The number of sex comb teeth and the metatarsal length were correlated ($r = 0.29^*$). With a larger sample, undoubtedly covariance would be found between more of the characters.

In the other genotypes a high degree of correlation existed between the number of sex comb teeth, the length of the tarsus, and the number of tibial or tarsal rows for most of the genotypes (Table 3). Furthermore, three variates (tibial to tarsal

TABLE 3

Correlation in expression of the extra sex comb characters in the second leg of males with one or more extra sex comb mutants

	esc/esc n=81	*Scx/+* n=88	*esc/esc; Scx/+* n=54	*Scx/Pc* n=50	*esc/esc; Scx/Pc* n=32
Sex comb teeth to length	0.40†	0.70†	0.48†	0.09	0.18
Sex comb teeth to tarsal rows	0.71†	0.63†	0.50†	0.32†	0.08
Sex comb teeth to tibial rows	0.71†	0.53†	0.22	0.22	0.22
Length to tarsal rows	0.39†	0.62†	0.39†	0.26	0.01
Length to tibial rows	0.17	0.43†	0.28†	0.39†	0.14
Tibial to tarsal rows	0.55†	0.61†	0.42†	0.41†	0.37*
Sex comb teeth to tarsal rows to tibial rows	0.71†	0.64†	0.41†	0.43†	0.42*

* Correlation coefficient (r) is significant at the five percent level.
† Correlation coefficient (r) is significant at the one percent level.

transverse rows to sex comb teeth) were correlated, even in the genotypes which had little correlation for two variates. Thus there is undoubtedly a mutual relationship in the expression of the characters in the extra sex comb genotypes.

The data also show that the expression of any two characters is not perfectly correlated. (In perfect correlation $r = 1.00$ and no correlation $r = 0.00$). In these genotypes (with significant r values between 0.32 and 0.71) there is considerable variation in expression of the characters in each leg. For example in the second legs of Scx males, the range in length of the tarsus in flies with no sex comb teeth was 68–72 units; 0–2 teeth, 65–72 units; 3–5 teeth, 62–73 units; 6–8 teeth, 61–69 units and 9–11 teeth 62–69 units (see Table 2 for mean values). In the same genotype 23 of the legs had no tarsal transverse rows yet had from one to five sex comb teeth, 25 legs with no tibial transverse rows had from one to nine sex comb teeth, and 12 legs had tibial but no tarsal rows and 13 legs had tarsal but no tibial rows. Thus there is considerable individual variation in expression of the characters (see also Figure 2, particularly E–H). A similar range in expression of the characters occurred in all single-factor genotypes. Among the 273 legs from Pc, esc and Scx, 66 legs had from one to six sex comb teeth but no tarsal transverse rows, or 60 legs had from one to seven teeth but no tibial row. On the other hand only five legs had tarsal or tibial rows without sex comb teeth. In spite of this great difference in expression, there was no evidence that the deviations are any greater than would be expected by chance. In the two- and three-factor genotypes the

range in expression of the bivariates was not as great: all flies had every character expressed to some degree, the variance for each character was less than in the single-factor genotypes and the means were close to the expected means characteristic of the first leg.

The reason for lack of correlation in five of the six tests for covariance between two characters in the esc-Scx-Pc genotypes (Table 3) becomes evident if the means for the characters in the second leg of the extra sex comb genotypes are compared to the means for the same characters in the first leg of Sam-Ore males (Table 1). The difference in means was significant (at 2 S.E.) for all 13 characters in the genotypes with a single extra sex comb factor; in each genotype the mean was closer to the mean of a second leg than a first leg. In the flies with either two or three extra sex comb factors the difference was significant for six of the 13 characters. However, in the flies with two factors the mean expression was intermediate between a second and a first leg, but in flies with three factors the mean expression for all characters was closer to a first leg than a second. Furthermore, the mean expression for four of the six characters with a significant difference (as compared to a first leg) made the second leg of esc-Scx-Pc flies more first leg than the first leg of Sam-Ore males: the metatarsus was shorter, there were fewer bristles in row 6, more bractless bristles in row 5.5 and more sex comb teeth. For all characters combined, the second leg of esc-Scx-Pc had become a first leg.

It is obvious from the data in Table 1 that a single extra sex comb factor is, on the average, not capable of transforming a second leg into a first leg. A portion of the flies, however, have changes which approach the mean for each character in the first leg (i.e., Scx in Table 2) and individual flies may show complete transformation of a second leg into a first leg. This range in expression of the extra sex comb factors in simplex may be because in each genotype a number of modifying factors are interacting with the primary factor to produce a wide range in the expression or it may mean that the genotypes are very sensitive to external or internal environmental conditions. Transformation of a second leg into a first leg in compounds of two factors is far greater than would be expected from their action singly, even if modifying factors are taken into consideration. For example, the mean incidence of sex comb teeth in Pc was 0.6 and Scx was 4.7, yet in the compound (Scx-Pc) the mean number of sex comb teeth was 11.2; or the number of tarsal transverse rows was none and 1.4 for Pc and Scx singly and 6.2 in the Scx-Pc compound (Table 1). The fact that the action of the extra sex comb factors is augmented in compounds as compared to their action singly is indicative of subthreshold effects of the extra sex comb factors, rather than modifying factors. If this is the case the factors singly contribute to variance in expression of the phenotype, but in compounds they interact to produce full expression of the extra sex comb characters.

The data also indicate that there is an upper threshold in expression of each character. For example, if the number of teeth per sex comb would be augmented in a three-factor compound to the same extent as it was in a two-factor compound, more than 12 sex comb teeth would be expected in esc-Scx-Pc males. The same is

true for the tibial and tarsal transverse rows (Table 1). Thus it seems likely that the extra sex comb factors, even when compounded, cannot transform a posterior leg beyond a certain point, that is to more than a first leg.

Metathoracic leg: Comparisons of the length of the metatarsus and the chaetotaxy of the metatarsus and distal end of the tibia of the male third leg for the seven genotypes are presented in Table 4 and examples of the various types in Figure 4. The data show that the extra sex comb factors cause the third leg to be

TABLE 4

Comparisons of the chaetotaxal pattern of the metatarsus and distal end of the tibia and the length of the metatarsus of the third leg of males from seven different genotypes

Genotype Sample	Sam-Ore 20/50	*Pc*/+ 27/102	*Scx*/+ 86	*esc/esc* 20/82	*Scx/Pc* 46/50	*esc/esc; Scx*/+ 20/53	*esc/esc; Scx/Pc* 16/32
Row 1	11.4±0.1	12.6±0.1	11.5±0.1	10.8±0.1	11.1±0.1	10.5±0.1	9.3±0.3
Row 2*	11.7±0.1	12.4±0.1	11.7±0.1	10.6±0.2	11.3±0.1	10.1±0.1	8.8±0.2
Row 3	10.9±0.2	10.6±0.1	9.7±0.1	9.6±0.1	9.2±0.1	9.0±0.2	8.9±0.3
Row 4	7.1±0.1	7.7±0.1	7.2±0.1	6.5±0.2	7.0±0.1	6.6±0.2	5.7±0.2
Row 5	6.6±0.1	7.3±0.1	6.9±0.1	6.2±0.2	5.9±0.2	5.8±0.2	4.9±0.3
Row 5.5	1.0±0	1.9±0.1	1.3±0.1	1.9±0.2	3.6±0.1	2.7±0.2	3.2±0.3
Row 6	7.1±0.1	7.2±0.1	6.9±0.1	6.2±0.2	5.2±0.2	5.6±0.3	4.2±0.3
Row 7	10.3±0.1	9.9±0.2	10.0±0.1	8.5±0.3	7.1±0.2	7.3±0.2	6.2±0.2
Row 8	12.9±0.2	14.1±0.1	13.0±0.1	12.2±0.2	11.6±0.2	10.8±0.4	7.8±0.3
Tarsus length	87.4±0.3	87.9±0.2	83.2±0.3	75.9±0.4	74.8±0.7	68.0±0.4	56.5±0.9
Sex comb teeth	....	0.2±0.1	0.5±0.1	2.9±0.3	8.3±0.4	8.1±0.5	11.8±0.3
Ta. tr. rows	....	0.0±0.0	0.1±0.0	0.8±0.1	3.2±0.2	2.6±0.3	6.1±0.2
Ti. tr. rows	....	0.0±0.1	0.0±0.0	1.1±0.2	3.3±0.2	4.0±0.2	5.0±0.1

* Row 2 lists the number of transverse rows.
The entries in each column include the mean±S.E. for 13 characters. The mean (and standard error) for the rows in Sam-Ore is based upon 20 legs, for the other characters upon 50 legs, or 27 legs and 102 legs respectively for *Pc*, etc.; the mean for each character in *Scx* is based upon a sample of 86 legs. For abbreviations and other details see Table 1.

changed into a first leg. However, transformation from a third leg to a first leg was not as complete for any one of the 13 characters in most of the genotypes as it was in the second leg.

The difference between the means, for each of the 13 characters, between the first leg of Sam-Ore males and Pc, Scx, esc, esc-Scx and Scx-Pc males was significant (at 2 S.E.) for all but one character (row 5.5 in Scx-Pc) with the extra sex comb genotypes always more third leg-like than first leg-like. The difference between the means of esc-Scx-Pc third legs and Sam-Ore first legs was significant for eight values. The greatest difference between the means was in the length of the tarsi and the number of bristles per row in rows 1–8. Even in these characters, however, there were changes making the third leg like a first leg. Of the characters normally not present in the third leg, i.e., sex comb teeth, tarsal and tibial transverse rows and more than one bractless bristle in row 5.5, only the tibial rows were significantly less in esc-Scx-Pc third legs than in Sam-Ore first legs. Thus it seems that for these characters the extra sex comb factors are almost as effective

in changing a third leg to a first leg as there are in changing a second leg into a first leg.

In spite of the difference in action in the two legs, the extra sex comb mutants appear to transform the whole leg, whether it be a second or a third leg, into a first leg not just to induce the formation of a sex comb. If all of the genotypes are

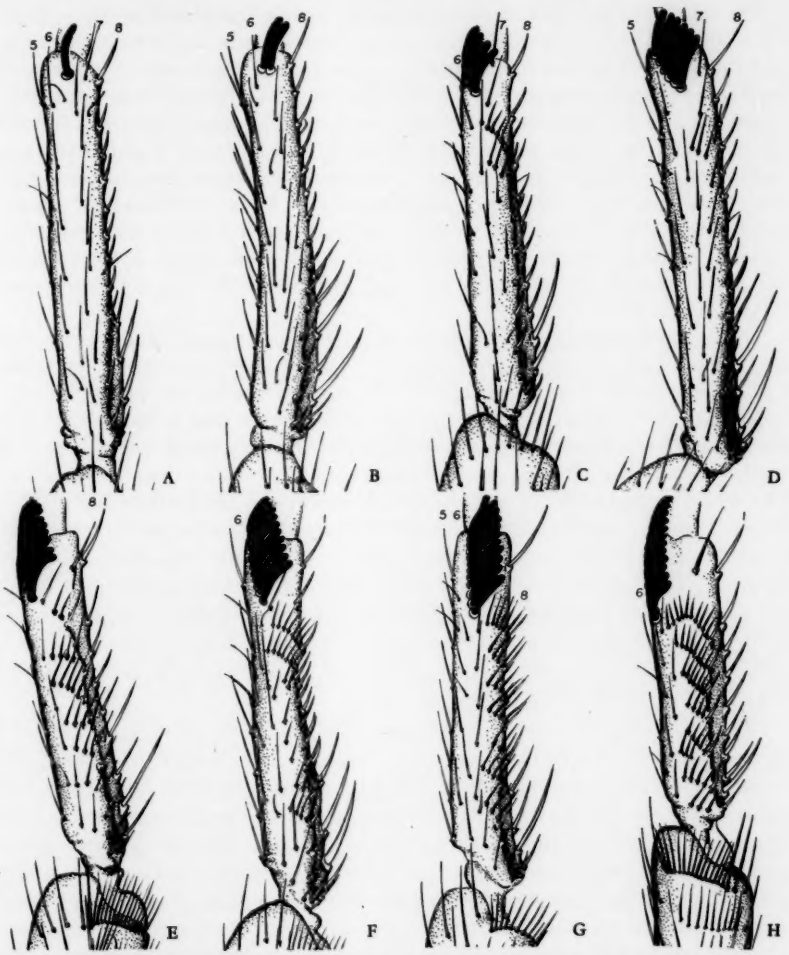

FIGURE 4.—Stages in transformation of a male third leg into a first leg through changes in size of the tarsus and chaetotaxy of the tarsus and tibia. A-H—Examples of the metatarsus and distal end of the tibia from third legs of males with one or more extra sex comb factors (A-B from Scx, C, E, G, and H from esc-Scx and D and F from Scx-Pc). For details see Figures 1A and D and 2.

ranked (taking into consideration the variance as before) for the 13 characters of the third leg the mean ranks place the genotypes in a graduated series:

$$\text{Sam-Ore} > \text{Pc} > \text{Scx} > \text{esc} > \text{Scx-Pc} = \text{esc-Scx} > \text{esc-Scx-Pc}$$

and the linearity is correlated with the increase in number of extra sex comb factors (Figure 3).

The difference in order of the mean ranks among the genotypes showed that the mode of action of the extra sex comb mutants must be different in the second and third legs. The greatest difference in the second leg occurred between the one- and two-factor compounds; in the third leg the difference was as great between esc and Scx, and the two- and three-factor compounds as it was between the one- and two-factor compounds. Secondly, the order of Scx and esc were reversed in the third leg as compared to the second leg (second leg—mean rank of Scx = 3.35, and esc = 2.88; third leg—mean rank of Scx = 2.58 and esc = 4.19). Furthermore, the difference between Scx and esc in transforming the two legs into first legs was expressed to the greatest degree in those characters which normally are not present in the posterior legs, e.g., the sex combs and transverse rows (Tables 1 and 4).

The difference between esc and Scx is shown most clearly in the penetrance and expressivity of the sex comb character. In Scx, 80.7 percent of the second legs had sex combs with an average of 5.9 teeth per comb in the legs with combs, while only 22.1 percent of the third legs had sex combs with a mean of 2.1 teeth per comb. This means that fewer third legs had sex combs and of those legs with combs, the number of teeth per comb was less in the third leg than the second leg. Therefore, both penetrance and expressivity are lower in the third leg than the second leg. In esc 92.7 percent of the second legs had combs with 5.9 teeth per comb on the legs with combs and 70.7 percent of the third legs had combs with 4.0 teeth per comb. In Pc 38.5 percent of the second legs had combs with 1.6 teeth per comb and 14.7 percent of the third legs had combs with 1.1 teeth per comb. Thus in the third legs of esc and Pc, penetrance is more affected than expressivity. Scx behaves more like esc in expression of the sex combs in the second leg, but more like Pc if the third leg is considered.

(1) *Systematic variation within a genotype:* Complete chaetotaxal studies of the tarsus and distal end of the tibia using relatively large samples were made only for two genotypes, Scx and Scx-Pc (Table 5). The third leg of Scx flies, if separated either on the basis of those with sex combs vs. those without sex combs, or by other criteria (as in Table 5) show little systematic variation. This could be interpreted as lack of correlation in expression of the various characters. However, if only the characters that are not normally present in the posterior legs are considered, there is some evidence for a systematic change within the Scx genotype.

In the Scx-Pc males the evidence of a systematic change in the third leg is more convincing although even in this genotype only nine of the 13 characters showed the expected increase or decrease parallel with an increase in number of sex comb teeth, in spite of the fact that the number of teeth per comb ranged from 0–15.

TABLE 5

Evidence of a systematic change in the expression of 13 characters in the third leg and in one- and two-factor genotypes

Genotype	*Scx/+*				*Scx/Pc*		
Phenotype Sample	No sex combs in 2 & 3 8	3rd leg not affected 39	Transverse rows in tarsus 20	Sex comb teeth 19	0–6 sex comb teeth 9	7–9 sex comb teeth 24	10–15 sex comb teeth 13
Row 1	11.6	11.5	11.6	11.3	11.7	10.8	11.2
Row 2*	11.9	11.7	11.7	11.6	11.7	11.0	11.5
Row 3	9.5	9.7	9.7	9.8	9.0	9.3	9.1
Row 4	7.1	7.3	7.2	7.2	7.0	7.0	7.0
Row 5	6.6	7.0	6.9	7.0	6.4	5.8	5.5
Row 5.5	1.1	1.2	1.3	1.7	3.4	3.6	3.6
Row 6	6.8	7.0	6.9	6.7	6.1	5.0	5.0
Row 7	10.3	10.2	10.0	9.7	7.3	7.3	6.5
Row 8	13.5	14.1	13.8	13.8	13.0	11.7	10.5
Tarsus length	83.1	83.5	83.8	82.1	78.9	74.5	73.7
Sex comb teeth	0.0	0.0	0.0	2.1	4.2	8.2	11.5
Ta. tr. rows	0.0	0.0	0.1	0.1	2.1	3.1	4.6
Ti. tr. rows	0.0	0.0	0.0	0.1	2.4	3.8	3.7

* Row 2 lists the number of transverse rows.
The 86 legs of *Scx* male were divided into four groups based upon phenotypic changes in the third leg. The 46 legs of *Scx-Pc* males were separated into three groups, 0–6, 7–9 and 10–15 sex comb teeth in the third leg. For details see Tables 1 and 2.

Such results are indicative of a large variance in the expression of each of the characters.

(2) *Covariance:* Only 15 of the 139 legs from Pc males had evidence of a chaetotaxal change, 14 with one or two sex comb teeth and one with a tibial row but no sex comb teeth. In Scx, 67 of the 86 legs were without combs; in spite of this the number of teeth was correlated with the length of the tarsus ($r = 0.45^{\dagger}$). Five males had transverse rows: one leg with four sex comb teeth had a tibial and a tarsal row, one leg, also with four teeth had only a tibial row; one leg with two teeth had a tibial row, one leg with one tooth had a tarsal row and one leg without any teeth had a tarsal row. The mutual relationship in the expression of the characters in the third leg becomes evident, by correlation tests, in the other genotypes. A high degree of correlation was found between two variates (six comparisons) for esc, esc-Scx and Scx-Pc, and a greater degree of correlation in esc-Scx-Pc in the third leg than in the second leg (Table 6). A significant correlation in Scx-Pc for all six pairs in the third leg and only three pairs in the second leg (Table 3) is further proof that there is an upper limit in the expression of each character. This threshold is reached for most of the characters in the second leg but not the third leg in Scx-Pc. In esc-Scx-Pc the threshold is reached for most characters, particularly the tibial rows, in both legs. This is expressed as a lack of correlation in either bivariate or trivariate comparisons.

As in the second leg, transformation of a third leg into a first leg in compounds of two factors, or three factors, is far greater than would be expected from their actions singly (Table 4). This is seen most clearly in the tarsal transverse rows:

in single factor genotypes there is less than one row, in two factor compounds the average is three rows and in the three factor compound the average is six rows. Thus each of these mutants has subthreshold effects which are only expressed in compounds with one another.

B. Changes in the metatarsus and tibia of the female meso- and methathoracic legs

The changes from the female second or third leg into a first leg include: (1) a decrease in the length of the metatarsus and a decrease in the number of bristles per row; (2) induction of transverse rows of bristles on the anterio-ventral and ventral surfaces of the metatarsus and distal end of the tibia. As these changes are not as obvious as the changes in the male, such as formation of a sex comb, it had formerly been thought that the extra sex comb mutants were sex-limited in their expression. However, the transformation of the posterior legs into female first legs occurs in the same manner as in the male and the final results are just as profound.

TABLE 6

Correlation in expression of the extra sex comb characters in the third leg of males with one or more extra sex comb mutants

	esc/esc n=82	*esc/esc; Scx/+* n=53	*Scx/Pc* n=50	*esc/esc; Scx/Pc* n=32
Sex comb teeth to length	0.25*	0.48†	0.39†	0.23
Sex comb teeth to tarsal rows	0.73†	0.64†	0.55†	0.51†
Sex comb teeth to tibial rows	0.50†	0.39†	0.48†	0.24
Length to tarsal rows	0.25*	0.37†	0.34*	0.62†
Length to tibial rows	0.13	0.08	0.29*	0.00
Tibial to tarsal rows	0.52†	0.27*	0.46†	0.01
Sex comb teeth to tarsal rows to tibial rows	0.65†	0.39*	0.54*	0.26

* The correlation (r) is significant at the five percent level.
† The correlation (r) is significant at the one percent level.

Mesothoracic leg: Comparisons of the length of the metatarsus and chaetotaxy of the metatarsus and distal end of the tibia of the female second leg for the seven genotypes are presented in Table 7 and examples of types in Figure 5. The principal change was in the length of the metatarsus and the transverse rows on the tibia and tarsus, which as in the male, changed proportionately with the increasing number of extra sex comb factors. If all of the characters are ranked (in the same manner as before) the mean ranks form a graduated series for the genotypes:

$$\text{Sam-Ore} > \text{Pc} > \text{esc} > \text{Scx} > \text{Scx-Pc} = \text{esc-Scx} > \text{esc-Scx-Pc}$$

The seriation of genotypes is the same as for the male second leg and consistent with the increasing number of extra sex comb factors (Figure 3).

The difference between the female and the male, e.g., the higher mean rank in the female for Sam-Ore, Pc, esc and Scx, and the lower mean rank for the female in the compounds may be because of sexual dimorphism in expression of the

TABLE 7

Comparisons of the chaetotaxy of the metatarsus and distal end of the tibia and length of the metatarsus of the first leg of Sam-Ore females and second legs from females of seven different genotypes

Genotype Sample	Sam-Ore 20		*Pc*/+ 20	*Scx*/+ 20	*esc*/*esc* 20	*Scx*/*Pc* 20	*esc*/*esc*; *Scx*/+ 20	*esc*/*esc*; *Scx*/*Pc* 6
	1st leg	2nd leg						
Row 1	8.0±0.0	11.6±0.1	12.6±0.2	11.5±0.3	11.1±0.2	10.0±0.1	10.0±0.2	9.2±0.4
Row 2	7.2±0.2	10.5±0.2	10.7±0.2	10.1±0.3	10.3±0.1	7.8±0.2	8.9±0.2	8.0±0.3
Row 3	5.7±0.2	6.9±0.1	7.4±0.2	6.9±0.2	7.7±0.1	6.6±0.1	6.7±0.2	6.0±0.0
Row 4	4.3±0.1	6.1±0.1	6.2±0.1	5.5±0.2	5.7±0.1	5.2±0.1	5.4±0.1	5.2±0.2
Row 5	4.7±0.2	6.1±0.1	6.1±0.1	6.0±0.1	5.9±0.1	5.2±0.1	5.4±0.1	5.0±0.0
Row 5.5	1.0±0.0	1.0±0.0	1.1±0.1	1.0±0.0	1.0±0.0	1.2±0.0	1.0±0.1	1.0±0.0
Row 6	5.4±0.1	7.0±0.2	6.7±0.1	6.7±0.2	7.2±0.1	6.1±0.1	6.1±0.1	5.2±0.2
Row 7	7.1±0.2	9.5±0.1	10.3±0.2	9.0±0.2	10.2±0.1	7.1±0.1	7.5±0.2	6.8±0.2
Row 8	8.1±0.1	11.8±0.2	12.2±0.2	11.7±0.3	11.5±0.2	9.3±0.2	8.7±0.2	8.2±0.2
Tarsus length	54.1±0.4	79.0±0.5	76.2±0.8	69.8±1.1	69.8±0.9	62.9±0.4	54.8±0.4	53.5±0.6
Ta. tr. rows	7.9±0.1	....	0.5±0.2	2.9±0.8	0.5±0.3	8.4±0.3	7.8±0.4	8.3±0.2
Ti. tr. rows	6.0±0.1	....	1.4±0.3	2.6±0.5	0.8±0.3	5.7±0.1	5.6±0.2	5.8±0.1

The entries in each column include the mean±1 S.E. for 12 characters: rows 1–8, tarsal transverse rows, tibial transverse rows, and length of the tarsus. For abbreviations and other details see Table 1 and Figure 1B and C.

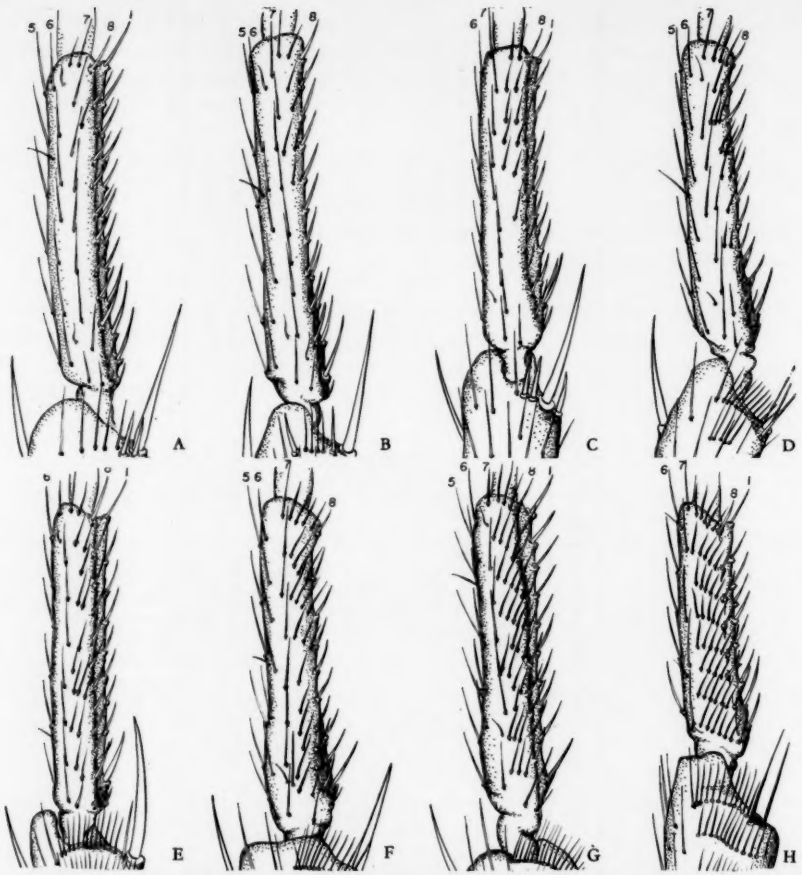

FIGURE 5.—Stages in transformation of a female second leg into a first leg through changes in size of the tarsus and chaetotaxy of the tarsus and tibia. A-H—Examples of the metatarsus and distal end of the tibia from second legs of females with one or more extra sex comb factors (B from Pc, A, E, and G from Scx; C, D, and F from esc; and H from esc-Scx-Pc). The newly induced or transformed bristles of the transverse rows are in solid black; the bristles characteristic of the second leg are in outline. For details see Figures 1B and C.

characters, or it may be due only to sampling error. A difference between the female and male in the expression of certain of the characters is suggested from comparison of individual characters in the two sexes.

The length of the metatarsus of the second leg decreases from approximately 77 to 45 units in the male while in the female the decrease is only from 79 to 54 units (Tables 1 and 7). In the first legs of Sam-Ore, the difference between the

two sexes in the length of the metatarsus is about 12 percent. In the second leg the difference between Sam-Ore females and males is only three percent while the difference between the second leg of esc-Scx-Pc females and males is 15 percent. The tarsus length of the female is reduced in size, as compared to Sam-Ore, by 32 percent and the male by 42 percent. This difference in decrease in length of the metatarsus in the two sexes is undoubtedly one of the reasons for the number of bristles per row, in rows 1–8, showing a greater decrease in the males than the females.

The bractless bristles (row 5.5) do not increase proportionately with the increase in number of extra-sex comb factors in the female. This would be expected if the extra sex comb mutants were sexually dimorphic in expression, for row 5.5 in the female first leg never has more than one or two bristles.

Except in esc, the number of tibial and tarsal transverse rows is usually greater in the female than in the male posterior legs of extra sex comb flies especially in the one- and two-factor genotypes. Such a difference would be expected for the tarsal rows because of the sexual dimorphism in number of rows in the first leg—an average of six rows in the male and eight in the female. In the tibia, however, both the female and male first legs had an average of six tarsal rows, thus a consistently higher number of rows would not be anticipated in the female posterior legs of the extra sex comb genotypes.

This dimorphism in expression of the various characters probably accounts for the higher mean rank of the females in the one-factor genotypes and the male in the three-factor genotype, with the females being higher in some but not all of the two-factor genotypes.

Metathoracic leg: Comparisons of the length of the metatarsus and the chaetotaxy of the metatarsus and distal end of the tibia of the female third leg are presented in Table 8 and Figure 6. As was found previously the changes in the expression of the extra sex comb phenotypes were proportional to the number of factors which when ranked form a graduated series for the genotypes:

Pc > Sam-Ore > Scx > esc > esc-Scx > Scx-Pc > esc-Scx-Pc

The seriation in the third leg of the females differs from the seriation in the second leg of both sexes and the third leg of the males (Figure 3). However, as the general pattern is the same as for the male third leg, the differences can probably be accounted for, to a large extent, by sampling error.

The most pertinent fact from ranking the genotypes is that in the female, as in the male, the rank of Scx and esc is reversed in the third leg as compared to the second leg. This means that in the females as in the males esc and Scx have different modes of expression in the second and third legs.

Finally, as was also found for the males, the threshold for expression of the extra sex comb phenotype was virtually reached in the second leg in two-factor compounds, but not until the three-factor compounds in the third leg.

C. Changes in the coxa and femur

In the wild type fly, the three pairs of legs differ considerably in the shape and structure of the coxa (Figures 7 A-C). The coxa of the first leg is more tubular,

TABLE 8

Comparison of the chaetotaxal pattern of the metatarsus and distal end of the tibia and length of the metatarsus of the third leg of females from seven different genotypes

Genotype Sample	Sam-Ore 20	*Pc*/+ 20	*Scx*/+ 20	*esc/esc* 20	*Scx/Pc* 20	*esc/esc*; *Scx*/+ 20	*esc/esc*; *Scx/Pc* 6
Row 1	11.6±0.2	12.3±0.1	11.6±0.1	10.4±0.2	11.2±0.2	10.5±0.2	9.8±0.3
Row 2*	11.8±0.1	12.4±0.2	11.7±0.1	10.5±0.1	11.1±0.1	10.4±0.1	10.0±0.3
Row 3	10.5±0.2	11.1±0.1	10.2±0.1	9.8±0.2	9.6±0.1	10.1±0.1	9.0±0.5
Row 4	7.4±0.1	7.9±0.1	7.9±0.2	7.1±0.2	7.3±0.1	7.1±0.2	7.2±0.3
Row 5	7.1±0.1	7.7±0.1	7.3±0.1	6.1±0.1	6.8±0.1	6.3±0.1	6.0±0.3
Row 5.5	1.0±0.0	1.4±0.1	1.0±0.0	1.0±0.0	1.1±0.1	1.0±0.0	1.0±0.0
Row 6	7.5±0.1	7.7±0.2	7.3±0.2	7.3±0.2	6.9±0.1	7.0±0.2	6.7±0.2
Row 7	10.5±0.2	10.7±0.2	10.0±0.3	10.3±0.2	8.4±0.2	9.5±0.2	8.3±0.5
Row 8	12.9±0.2	14.4±0.2	13.9±0.3	12.9±0.2	11.7±0.2	12.6±0.4	11.0±0.5
Tarsus length	90.6±0.4	89.3±0.5	85.7±0.4	75.5±1.0	77.9±0.5	74.3±1.2	65.0±1.7
Ta. tr. rows	....	0.3±0.1	0.4±0.2	0.3±0.1	7.1±0.4	3.2±0.5	7.3±0.8
Ti. tr. rows	....	0.8±0.3	0.4±0.2	0.3±0.2	4.8±0.1	3.4±0.4	4.8±0.3

* Row 2 lists the number of transverse rows.
The entries in each column include the mean±1 S.E. for 12 characters. For abbreviations and other details see Table 1.

longer and more separated from the body than the coxae of the posterior legs. The principal difference in the femurs is in the complete proximal-distal row of larger, longer bristles on the posterio-dorsal surface of the femur of the first leg but absent in the posterior legs.

The coxae and femurs of the posterior legs were transformed into first leg-like segments only in compounds of several extra sex comb mutants. Transformation occurs in the same manner and is parallel with transformation of the tibiae and metatarsi (Figures 7 D-G). However the changes are not as great as in the more distal segments. The legs of both the female and male are affected to the same extent by the factors or their compounds.

Scx and Pc: The flies of these two genotypes have little if any changes in the structure or chaetotaxy of the coxa or femur of either posterior leg indicative of transformation from a second or third leg into a first leg.

esc: The coxae of neither the second or third leg show any changes indicative of transformation of these segments into first leg segments. The femur usually has the characteristic chaetotaxy of the second or third leg. Not infrequently, however, a row of bristles on the posterio-dorsal surface of the mesothoracic femur is more distinct and the bristles are somewhat longer. There is no evidence of two or three longer preapical bristles on the dorsal surface of the femur.

Scx-Pc: The coxae of both the second and third legs had very slight morphological changes, if any, in the direction of the first leg. Many flies—even those with profound changes in the metatarsus—show no evidence of a row of bristles on the posterio-dorsal surface of the femur. In others, two or three bristles are in the proper position and long enough to be considered evidence of a chaetotaxal change in the direction of a first leg. In a very few flies the row of longer bristles is complete and not distinguished from the row of bristles in the first leg.

esc-Scx: The coxa of the second leg shows considerable transformation toward a first leg coxa so that in the majority of the flies it is intermediate in structure and chaetotaxy between the first and second leg. The row of bristles on the posterior-dorsal surface of the femur may be entirely missing or present to a varying

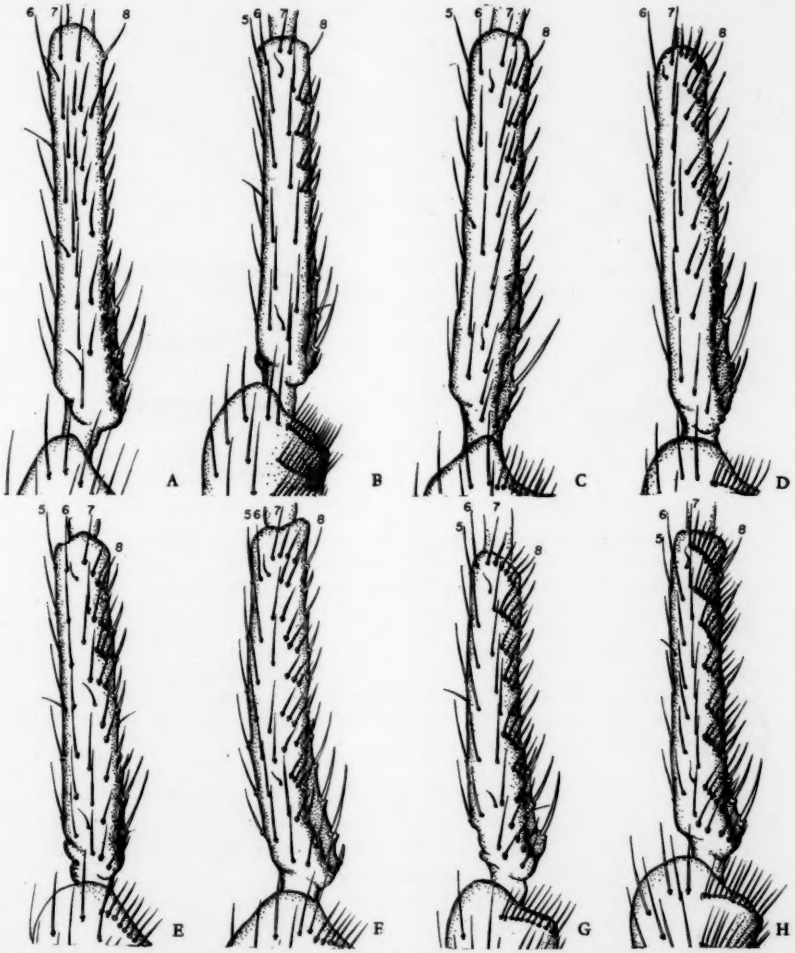

FIGURE 6.—Stages in transformation of a female third leg into a first leg through changes in size of the tarsus and chaetotaxy of the tarsus and tibia. A-H—Examples of the metatarsus and distal end of the tibia from third legs of females with one or more extra sex comb factors (A and B from Scx; C from esc; D and F from Scx-Pc and E, G, and H from esc-Scx-Pc). For details see Figures 1B and D and Figures 5.

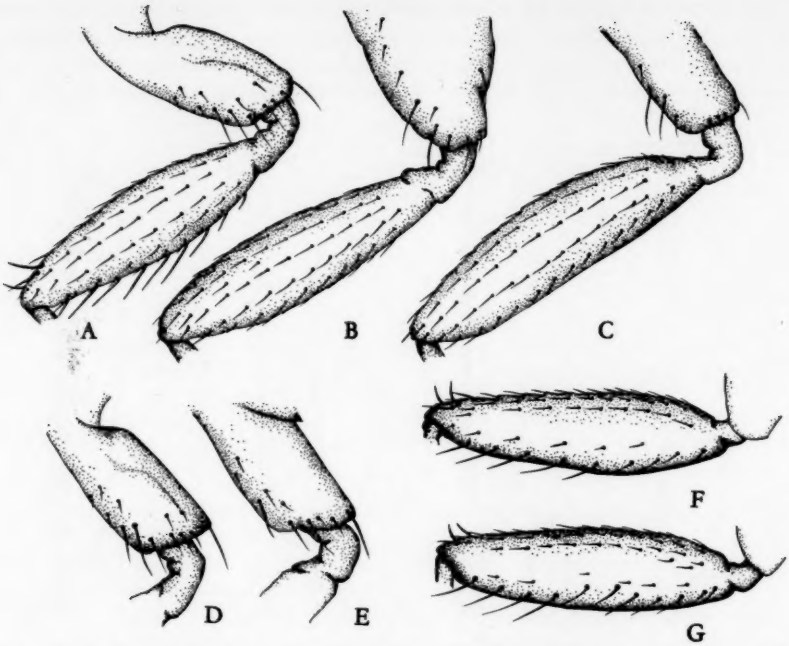

FIGURE 7.—Drawings of the most proximal segments of the three legs of Sam-Ore and extra sex comb males depicting transformation of the coxa and femur of the second leg into coxa and femur characteristic of the first leg. A.—Anterio-lateral view of the coxa and femur of a first leg of a Sam-Ore male showing the structure and chaetotaxy of the coxa and the complete row of bristles on the posterio-dorsal surface of the femur. B.—Same for the second leg. The complete row of bristles is absent from the femur. C.—Same for the third leg. D.—The coxa of a first leg from an esc-Scx-Pc male (anterior view). E.—A second leg from the same fly showing transformation of the coxa into a first-leg coxa. F.—The femur of a first leg from a Scx-Pc male (dorsal view). G.—The femur of a second leg from the same fly showing the row of bristles on the posterio-dorsal surface of the femur (dorsal view).

degree. However, the group (usually three to four) of longer bristles on the dorsal surface is, in the majority of the legs examined, well differentiated. The coxa of the third leg has less change than the coxa of the second leg, and there is never any indication of rows of bristles on the femur.

esc-Scx-Pc: The changes in the coxa of the second leg are more profound than in esc-Scx flies. The coxa is frequently like a first leg coxa (Figure 7 E), but even in the flies with the greatest transformation in the direction of the prothoracic segment, it is only about 60 percent as long as the first leg coxa and never as well separated from the body. The majority of the flies have a complete row of bristles on the posterio-dorsal surface of the femur of the second leg and frequently the group of longer preapical bristles on the dorsal surface (Figure 7G). The third

legs of esc-Scx-Pc flies show little indication of morphological changes in the coxa and femur indicative of transformation of these segments into first leg segments.

It may be that the crippling of the legs, which occurs in many of the esc-Scx-Pc flies, is partially responsible for some of the morphological changes interpreted as homoeotic changes. However, as the observations were made primarily on legs which were not crippled, it seems reasonable to conclude that the changes in the coxa and femur of the posterior legs of esc-Scx-Pc flies are homoeotic changes just as the sex combs and transverse rows are homoeotic changes. Furthermore, it seems reasonable to assume also that the changes in the posterior legs of esc-Scx-Pc flies are the result of the transformation of these legs into first legs, just as they are in the two-factor compounds where leg-crippling was not encountered.

DISCUSSION

The sex combs are taxonomically important morphological structures in many but not all of the species of the Sophophora subgenus of Drosophila. In some species they occur only on the metatarsus, in others they are on both the first and second tarsal joints of the male first legs. A great variety of types of combs and range in numbers of teeth per comb occur in all of the species-groups (c.f., Figure 10, STERN 1954b). SPIETH (1952) proposed that the sex combs function as claspers during courtship and mating, and pointed out the concordance in type of comb and type of mating behavior. There is little doubt that sex combs are of selective value and it can be presumed that they have been derived from an ancestral form or ancestral forms. The different types could arise either by a slow elaboration of new genetic systems or by more sudden changes initiated by one or a very few genetic changes.

It is well known that the number of teeth per comb in *D. melanogaster*, as well as in other species, varies from strain to strain and that sometimes, but not always, there is a response to selection (c.f., BRASTED 1941; MATHER and DOBZHANSKY 1939; PREVOSTI 1955; SLIFER 1942). On the other hand, many mutants are known which in one step reduce the comb to one or two teeth, change the whole morphology of the comb or induce the formation of new combs (c.f., BRIDGES and BREHME 1944; HANNAH and STRÖMNAES 1955; STERN 1954b; STRÖMNAES unpublished; STURTEVANT 1929). If a single mutant can have such drastic effects upon differentiation of the sex comb, "it seems entirely possible that the evolutionary processes which diversified the sex comb features in different species began with the same kind of sudden changes . . . namely the response of mutated genes to pre-existing developmental prepatterns and the creation of new prepatterns by mutations." (STERN 1954b).

The concept of prepatterns was first proposed by STERN and HANNAH (1950) to interpret differentiation of the sex combs in gynanders of *D. melanogaster*, and has been further developed by these authors in studies of both bristles and sex combs (HANNAH-ALAVA and STERN 1957; STERN 1954a, b, 1956a, b, 1957; STERN and SWANSON 1957). From such studies it was concluded that there are at least

two ways in which genes may determine the bristle patterns. The genes may affect the paths of development in such a way as to bring about, early in development, different over-all organization of a region, i.e., different prepatterns in different genotypes. Or the differential effect of different genes may not set in until after the establishment of a given, unchanged prepattern. In other words, "in the first alternative different bristle patterns would originate in consequence of different prepatterns; in the second, the bristle pattern would depend upon the genetically controlled competence of the hypodermal cells to respond or not to respond to the singularities of the constant prepattern." (STERN 1957).

Many of the mutants of Drosophila can be classified as either "prepattern" or "terminal-pattern" mutants. Among the many mutants which remove bristles, most are probably of the latter type; that is, the prepattern for development of a bristle in a specific position is present but for some reason the bristle does not develop. The mutant *split*—in which the bristles are sometimes doubled and sometimes missing—is probably an example of a terminal-pattern mutant and also an example of the complex morphogenetic relationships in differentiation of the phenotype. Histological studies by LEES and WADDINGTON (1942) have shown, firstly, that three instead of two divisions occur, producing four instead of two cells at each bristle site and secondly, that orientation of this group of four cells in the hypodermis determines whether no bristle, one bristle or two bristles are finally differentiated. In one split strain, bristles are rarely formed because most of the groups of four cells lie below the surface of the epithelium and do not develop into bristles. Obviously the prepattern for development of a bristle at each bristle site is presented in split flies, but the final pattern is dependent upon a second morphogenetic process—the position and orientation of the presumptive bristle cells.

The homoeotic mutants which change the development of a segmental appendage into another type are probably the best example of prepattern mutants. Among the homoeotic mutants several are known which change the legs into different types or even transform other completely different organs into legs: *aristapedia* (BALKASCHINA 1929) in which the arista becomes a tarsus, *Antennapedia* (LEWIS 1956) in which the antennae becomes a leg, *bithorax* (BRIDGES and MORGAN 1923) in which the third leg in some respects becomes a second leg (along with other changes), *bithoraxoid* (LEWIS 1951) in which an abdominal third leg is formed (as well as other changes) and the extra sex comb mutants which change the posterior legs into first legs.

In understanding the results from the extra sex comb genotypes and their application in analysis of prepattern mutants, several important questions are raised: Why are sex combs differentiated only on the first leg and not on the second and third legs of normal flies? How do the extra sex comb genotypes cause the differentiation of sex combs in legs not normally possessing them?

If it is assumed, for example, that the prepattern for sex combs is already present in all of the legs but that "intensity" (c.f., STERN 1954b) is the deciding factor in the expression of the final phenotype, the lack of sex combs on the pos-

terior legs could be interpreted as an intensity below the threshold of expression in these legs. According to this hypothesis the extra sex comb genotypes would respond to lower intensities than the non sex comb genotypes. Another interpretation is that a diffusible "sex comb substance" produced somewhere in the body is responsible for the formation of the sex combs (c.f., BRASTED 1941; SLIFER 1942). According to this hypothesis, an increase in the sex comb substance in the extra sex comb flies would induce the formation of sex comb teeth in the posterior legs. Either a gradient in the substance itself or a gradient in threshold of response in the legs would account for lack of combs in the posterior legs of normal flies and their presence in extra sex comb flies.

Both hypotheses are based, in part, upon the assumption that only the sex combs are the product of the action of the extra sex comb mutants. Such schemes would not account for the homoeotic effects of the extra sex comb mutants, for they transform a second or third leg into a first leg and all segments are affected. Neither would such schemes explain the interactions of *Antennapedia*, *aristapedia* and the *extra sex comb* mutants in the production of sex combs upon the antennal legs (see STERN 1954b for details). Thus it seems likely that the extra sex comb factors act by changing the prepattern of the embryonic legs, and to this changed prepattern the cells in the male genotype respond by formation of sex comb teeth.

SUMMARY

1. A morphological and chaetotaxal study was made of the legs of flies with one or more of the extra sex comb mutants, extra sex comb (*esc*) a second chromosome recessive, Polycomb (*Pc*) and Extra sex comb (*Scx*) two homozygous lethal third chromosome dominants. Comparisons were made between seven genotypes: wild type, *esc/esc*, *Pc/+*, *Scx/+*, *esc/esc;Scx/+*, *Scx/Pc* and *esc/esc;Scx/Pc*.

2. The primary action of each mutant is to change the posterior legs into first legs by changes in the size, shape and chaetotaxy of all of the segments. Although there was a considerable range in expression of each of the characters, a change in any one character was accompanied by a proportional change in another character, that is the expression of the characters are covariant.

3. The most conspicuous character in the extra sex comb genotypes is the sex combs on the metatarsi of the second and third legs of the males. These mutants also induce in both sexes transverse rows of bristles on the distal end of the tibia and metatarsus and a complete row of bristles on the femur, characters normally present only in the first legs. In addition there are changes in the size, shape, and chaetotaxy of the coxae and in the number of bristles per row and the length of the metatarsi in the posterior legs.

4. The extent of change in the posterior legs is proportional to the number of extra sex comb factors. A complete transformation of a second leg into a first leg is achieved only in the compound of all three mutants. The augmented action of the mutants in compounds as compared to their action singly is indicative of subthreshold effects which in the single factor genotypes contribute to variance in

expression of the phenotype, but in compounds interact to produce full expression of the extra sex comb characters.

5. Transformation of a second or third leg into a first leg occurs in both sexes, but in the males the posterior legs become male first legs while in the females the posterior legs are transformed into female first legs.

6. The three mutants can be differentiated from one another by differences in penetrance, expressivity and action in the compounds.

7. From a consideration of the phenogenetics of the extra sex comb mutants and by comparison to other homoeotic mutants, it is proposed that they are "pre-pattern" mutants.

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ON THE RECOGNITION AND INHERITANCE OF THE SOLUBLE BLOOD GROUP PROPERTY "Oc" OF CATTLE[1]

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THE J system of bovine blood groups was first recognized by STORMONT (1947, 1949, 1952) and STORMONT, OWEN and IRWIN (1951) as a system independent of the systems A-H, B, C, F-V, S-U and Z. The J system has been regarded as a system composed of two principal phenotypes, namely, J-positive and J-negative, with evidence for a variety of intergrades in the J-positive category ranging from very weakly positive to very strongly positive. There has also been evidence to suggest that the various intergrades are controlled by distinct alleles in the *J* series but there is no direct test which will permit specific classification of the intergrades.

Unlike the other blood group systems of cattle, which of necessity have employed immune sera in their recognition and differentiation, the recognition and differentiation of the **J** phenotypes has been dependent solely on the use of natural isoantibodies (called anti-J) found in the serum of most J-negative cattle. The J system is unique in other ways, as pointed out by STORMONT (1949), but perhaps the most distinctive feature of this system is that the J substance is not an autonomuous product of the hematopoietic tissues. Whatever its ultimate chemical description, J seems to be primarily a soluble specific blood group substance (SSS) elaborated elsewhere in the animal body. This SSS makes its appearance in the blood plasma where some of it is absorbed onto the surface of the erythrocytes thereby making them susceptible to the hemolytic action of anti-J and complement. STORMONT (1949) demonstrated this acquisition of J-SSS in both *in vivo* and *in vitro* experiments. In the same report, he pointed out that the reactions of anti-J are not limited solely to J-SSS. On the contrary, anti-J specifically agglutinates human erythrocytes from group A and AB donors and is readily and completely absorbed by the A agglutinogen of man. Anti-J also reacts with SSS in the saliva and plasma of group A and AB secretors, and with J, R or A-like SSS in commercial preparations of pepsin and trypsin derived from cattle, sheep and swine. Indeed, all of these materials exhibited a greater avidity for anti-J than did J-SSS.

In 1951 STORMONT noted that erythrocytes of group R sheep are hemolysed by amounts of anti-J insufficient to cause hemolysis of the most strongly J-positive

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red cells of cattle. Use was then made of blood from group R and group O sheep in screening serum from J-negative cattle for the presence of anti-J and, in the course of such a survey, a serum was encountered which had the unique property of reacting with the red cells of group O (i.e., R-negative) sheep but not those from group R sheep (STORMONT, 1951). With the aid of this serum, called natural bovine antisheep O, he showed that group O is inherited as a recessive to R, an observation confirmed by RENDEL, *et al.* (1954) and RENDEL (1957) using antisheep O provided by this laboratory.

The bloods of numerous cattle (J-positive and J-negative) were tested with antisheep O and although none of the bloods were hemolysed by the action of that serum and complement, the author observed that the serum of certain J-negative and J-positive cattle possessed SSS capable of inhibiting the hemolytic action of antisheep O on the red cells of group O sheep. In spite of the fact that the red cells of such cattle were not lysed by the action of antisheep O and complement, they were, however, capable of absorbing all of the detectable anti-O hemolysins for sheep red cells. It was evident, therefore, that certain J-positive and J-negative cattle must possess a SSS related to O-SSS of sheep. This substance (or substances) is designated Oc (where O stands for "O-SSS" and c stands for "of cattle"). The present report is primarily concerned with a description of the **J-Oc** phenotypes in cattle and data relative to their inheritance.

MATERIALS AND METHODS

The results reported throughout this study were obtained from blood samples taken from Hereford cattle in the University of California beef herd and from other sources as noted.

Both "wet" and "dry" samples were collected. Wet refers to samples collected in isotonic citrate solution (two percent sodium citrate plus five percent sodium chloride) which acts as an anticoagulant. The wet samples were held at refrigerator temperatures and served as a source of erythrocytes for test suspensions and absorptions.

Dry refers to samples collected without anticoagulant. The blood coagulated soon after collection, and the clear serum was separated from the erythrocytes by centrifugation and stored at $-20°$ C for further use. Just prior to use, samples were thawed, prepared in appropriate dilutions and heated for 20 to 30 minutes at 56° C to inactivate complement.

Throughout this study the criterion for specific antigen-antibody reactions was the hemolysis of erythrocytes effected by the combined action of antibody and complement.

The principal antibodies used as standardizing reagents in this study were anti-J and antisheep O. The former reagent, a particularly potent and discriminatory J reagent, was obtained from a stock serum (C86) collected by STORMONT. This serum had a titer of approximately 1:1000 in tests on strongly reactive J-positive cattle erythrocytes and a titer of 1:2000 in tests on erythrocytes of group R sheep. Antisheep O reagent was the original serum (C45) collected by STOR-

MONT (1951) from a Hereford bull. This reagent had a titer of approximately 1:128 in tests on sheep erythrocytes of group O.

Complement obtained from guinea pigs was used in this study. Rabbit complement was found to be less satisfactory than guinea pig complement and was therefore not used. The complement usually consisted of a pool of serum from 7–20 animals. Only those sera were used which contained little or no natural antibodies reactive with cattle erythrocytes. The guinea pig complement was usually diluted between 1:10 to 1:20 for the tests. The exact dilution depended on the complement activity of the paricular aliquot.

The procedures used in the study of each sample of cattle blood were as follows:

Initially, a 2.5 percent suspension of thoroughly washed erythrocytes in 0.91 percent NaCl was prepared and tested in a standard cattle blood typing test after the methods of STORMONT *et al.* (1951). These tests served a twofold purpose: (1) as a preliminary classification for the J system and (2) as a check on the genetic compatibility of the blood groups of each individual with respect to those of its living corelatives.

The serum of each animal was tested for the presence of soluble J substance and Oc substance. Unabsorbed, heat-inactivated sera were used throughout these tests. The test employed in the detection of soluble substances was an inhibition test and was set up as follows:

To the first of a set of five 10 × 75 mm test tubes each containing 0.10 ml (2 drops) of C86 anti-J reagent diluted 1:20, was added 0.05 ml (one drop) of a 1:2 dilution of the serum being tested for J-SSS; to the second tube was added one drop of a 1:8 dilution of the serum; to the third tube was added one drop of a 1:32 dilution of the serum, to the fourth tube was added one drop of a 1:128 dilution of the serum and to the fifth tube was added one drop of a 1:512 dilution of the serum. The five tubes were shaken and allowed to stand 15 minutes at room temperature (22–27° C) at which time one drop of a 2.5 percent suspension of washed erythrocytes from a selected J-positive donor (cow No. 2) was added to each of the five tubes. The mixture of reagent, serum and red cells was shaken and allowed to stand ten minutes at which time one drop of diluted guinea pig complement was added to each tube. The tubes were again shaken and allowed to stand at room temperature. Hemolysis was recorded at approximately 30 minutes and again at 2–4 hours. (The particular dilution of C86 anti-J used in these inhibition tests was chosen as the most appropriate dilution for revealing inhibition of anti-J only after a series of extensive preliminary studies of the sera from a variety of J-positive donors ranging from strongly J-positive to weakly J-positive.)

To a second set of five 10 × 75 mm tubes each containing 0.10 ml of a 1:25 dilution of C45 anti-O reagent, precisely the same procedure was carried out on the serum being tested as that described in the foregoing paragraph, with one exception. To the mixture of the anti-O reagent and serum was added one drop of a 2.5 percent suspension of washed erythrocytes from a selected O-positive donor (sheep No. 999). These tests for the inhibition of anti-O were run in parallel

with the tests for inhibition of anti-J, and appropriate positive and negative controls as well as complement and saline controls were incorporated in all the tests. In many instances the results were repeatedly checked, especially in those cases where the inhibition of either anti-J or anti-O was slight or questionable.

If the serum of the individual being tested inhibited both anti-J and anti-O, that individual was classified as of phenotype or group **JOc**. If the serum inhibited anti-J but not anti-O, the individual was classified as group **J**. If the serum inhibited anti-O but not anti-J, the individual was classified as group **Oc**. If the serum failed to inhibit anti-J or anti-O, the individual was classified as group "—", where the dash (—) symbol stands for J-negative Oc-negative. No attempt was made in this study to account for or subdivide the various intergrades of **JOc**, **J** and **Oc**. In each of these three categories there was considerable variation in the degree of inhibition.

RESULTS

Table 1 shows the **J-Oc** classification of 379 offspring and their parents arranged into ten mating types. These data were tabulated from results of tests on the serum of University of California Herefords, registered Aberdeen Angus in a herd owned by MR. JOHN HERSHEY of Oildale, California, and Jersey cattle in a herd owned by MR. E. E. GREENOUGH of Merced, California.

Table 2 has been abstracted from Table 1 and shows the number of Oc-positive (including both phenotypes **JOc** and **Oc**) and Oc-negative offspring from the three possible kinds of matings. These data are with but three exceptions compatible with the view that the property Oc, as it occurs in phenotypes **JOc** and **Oc**, is inherited as a recessive character. Two of the exceptions may be due to faulty parentage information or difficulties in identification of the samples. They occurred as the result of the mating of a sire of type Oc with dams of type JOc to produce offspring of type J rather than JOc or Oc as would be expected if the

TABLE 1

J system phenotype classification of 379 offspring from known parental types

Parental phenotype	Offspring phenotype			
	JOc	**J**	**Oc**	**—**
JOc × **JOc**	19	1*	3	0
J × **JOc**	15	20	4	5
Oc × **JOc**	43	1*	25	1*
— × **JOc**	4	15	11	40
J × **J**	3	6	4	0
Oc × **J**	7	8	13	1
— × **J**	2	11	8	16
Oc × **Oc**	0	0	31	0
— × **Oc**	0	0	6	12
— × —	0	0	1	43

* Refer to text.

property Oc is truly a recessive. At present, the possibility that the two dams actually were misclassified as Oc-positive cannot be excluded. A possible explanation for the third exception is discussed elsewhere in this report.

Table 3 presents the **J-Oc** classification of all the data available on the three herds. Chi-square values, computed to determine whether or not there exists association between the J substance and the Oc substance, are given in Table 3. These values are interpreted as meaning that the J and Oc properties are related as the products of an allelic series.

A minimum of four alleles is proposed to account for the four phenotypic classes. The proposed alleles are J^{JOc}, J^{J}, J^{Oc}, and j. (If the various degrees of J and Oc were considered in detail, the number of alleles would, no doubt, be larger.) The phenotype-genotype relationships which are proposed are shown in Table 4. Gene frequency estimates for the J system in various breeds will be presented elsewhere.

TABLE 2

The classification with respect to Oc substance of 379 offspring from parents of known **Oc** *phenotype*

Mating types	Offspring phenotypes	
	Oc	non-**Oc**
Oc × **Oc**	121	3*
Oc × non-**Oc**	60	101
non-**Oc** × non-**Oc**	18	76

* Refer to text.

TABLE 3

Chi-square test for heterogeneity computed to test the independent occurrence of the J substance and Oc substance in three herds

	Number tested	**JOc**	**J**	**Oc**	—	χ^2	P
U. C. Beef	453	29	93	58	273	2.25	>.10 <.20
Aberdeen-Angus	224	88	66	67	3	33.67	<.001
Jersey	354	174	40	140	0	29.52	<.001

TABLE 4

Proposed phenotype-genotype relationships in the J-Oc system of bovine blood groups

JOc	**J**	**Oc**	—
J^{JOc} J^{JOc}	J^{J} J^{J}	J^{Oc} J^{Oc}	J^{Oc} j
J^{JOc} J^{Oc}	J^{J} J^{Oc}		j j
	J^{J} j		
	J^{JOc} J^{J}		
	J^{JOc} j		

DISCUSSION

Soluble elements of body fluids which are capable of reacting specifically with antibodies are commonly referred to as soluble specific blood group substances. However, when the specific meaning of the terms J substance or Oc substance is examined it is found that the implication of a separate or particular chemical entity carried by the word "substance" is not in fact intended. What is intended is simply the presentation of a phenotype. Thus, when the occurrence of animals of phenotype **JOc** is cited, it is not known whether there is one molecular substance which cross reacts with both anti-J and anti-O or more than one. Therefore the designations J substance and Oc substance are used here in their phenotypic rather than molecular sense.

The soluble blood group substances Oc and J of cattle are of considerable interest not only because of their relationship to anti-O and anti-J as discussed more fully in a following report (SPRAGUE 1958), but because of the general similarity exhibited between the J system of cattle and the R-O system of sheep, and the A-O system of swine. Each of the species has a substance (s) which cross reacts with anti-J as well as a substance (s) which cross reacts with anti-O.

In sheep, STORMONT (1951) and RENDEL *et al.* (1954) have shown that the blood group substance O (called "r" by RENDEL *et al.*) is inherited as a recessive character. Similarly the LEWIS (Le^a) blood factor is also a recessive blood factor (ANDRESON 1946). Likewise, the cattle Oc substance appears to be inherited as a recessive character as evidenced by the data presented in this report.

One exceptional Oc-negative (type —) offspring in Table 1 resulting from an **Oc** × **JOc** mating may illustrate a further correspondence between the J blood group system and the R-O blood system of sheep. RENDEL *et al.* (1954) have shown that sheep of type i (i.e., non-R non-O) are homozygous for a gene which suppresses the normal expression of the R-O phenotype. Sheep of type i may occur in various matings of R and O including O × O. It is therefore possible that the present exception may be due to a similar mechanism.

The chief difference between the J system and the R-O system are: (1) that "J" and "Oc" are found to co-exist in some sera, whereas R and O are not, and (2) that "Oc" on the red cells of cattle is demonstrable only by means of absorptions, whereas O on sheep erythrocytes is demonstrable by direct hemolytic tests as well as by absorptions.

Concomitant with the present study, the author tested the bloods of 217 swine with anti-J (the C86 serum) and anti-O (the C45 serum). It was observed that 94 of the bloods were hemolysed by anti-J (cattle anti-J is also antiswine A), 61 were hemolysed by anti-O, and 62 failed to react with either reagent, thereby establishing a relationship between the J-Oc system of cattle, the R-O system of sheep and the A-O system of swine (SPRAGUE 1955). Although no genetic or family data were available, the results clearly indicated the utility of natural bovine antisheep O in studies of the A-O blood group of swine.

In a following report (SPRAGUE 1958), data are presented relative to the distribution and inheritance of natural, bovine antisheep O.

SUMMARY

A soluble blood group property of some cattle sera detected only by inhibition tests is recognized; the property has been designated "Oc" and it is related both serologically and genetically to J. The Oc property appears to be inherited as a recessive character.

As a consequence of these observations, the J system of cattle has been extended to include four main phenotypes: **JOc, J, Oc,** and **—**.

A minimum of four alleles, namely J^{JOc}, J^{J}, J^{Oc}, and j have been proposed to account for the observed phenotypes.

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ON THE DISTRIBUTION AND INHERITANCE OF A NATURAL ANTIBODY IN CATTLE[1]

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THE origin of so-called normal or naturally occurring antibodies has provided a fascinating material for discussion almost since the beginning of immunology. There is ample evidence to indicate that many naturally occurring antibodies have arisen as a consequence of inapparent immunization, (BAILEY 1928), and certain investigators contend that all antibodies result only as a consequence of immunization (DUPONT 1934; WEINER 1951). Others propose that certain regularly occurring natural antibodies, as, for example, the isoagglutinins of man, are genetically conditioned (LANDSTEINER 1945; FURUHATA 1927). There have, until recently, been few if any situations involving natural isoantibodies which lend themselves to genetic analysis.

In 1951, STORMONT discovered that certain cattle possess natural hemolysins which act selectively on the red cells of group O sheep. He designated these hemolysins as natural bovine antisheep O. Serum samples from hundreds of cattle were screened for additional examples of antisheep O but none were found outside of the original family—a Hereford bull and three of his seven daughters. This suggested that natural, bovine antisheep O is conditioned by the action of a single dominant gene (STORMONT 1953). In the foregoing report it was shown that antisheep O also reacts with a soluble, specific blood group substance (s), called Oc, in the blood of certain cattle, thereby permitting in conjunction with the use of anti-J, the recognition of four phenotypes **J**, **JOc**, **Oc** and "—" in the J or J-Oc system of cattle (SPRAGUE 1958; SPRAGUE and STORMONT 1957). The present report is concerned with the influence of the phenotypes **J**, **JOc** and **Oc** of the J-Oc blood group system on the appearance of anti-O and on additional data bearing on the inheritance of natural, bovine antisheep O.

MATERIALS AND METHODS

Each sample of normal bovine serum was tested for the presence of both anti-J and antisheep O. The samples were diluted 1:4 and 1:16, based on a previously established optimum of 1:4 to 1:16, heated for 30 minutes at 56° C and were then tested with the red cells of four group R sheep, four group O sheep and one group i (i.e., non-R, non-O) sheep. Usually, but not invariably, J-positive and J-negative

[1] The results reported were taken from the author's dissertation submitted to the University of California in partial fulfillment of the requirements for the Ph.D. degree.

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cattle red cells were included in the tests to help serve as an indicator of the presence of anti-J but this was not a necessary procedure in view of the fact that anti-J has considerably greater avidity for the red cells of group R sheep than it has for those of J-positive cattle.

The tests were performed as follows: to 0.10 ml (two drops) of the diluted serum in a 10 × 75 mm test tube was added one drop of a 2.5 percent suspension of washed red cells of a given individual. This mixture was shaken and allowed to stand at room temperature (22 to 27° C) for 10 minutes at which time one drop of guinea pig complement (diluted 1:10 to 1:20) was added. The mixture of serum, red cells and complement was shaken and the first reading of the test was made approximately 30 minutes later. The tests were again shaken and a second reading was made after an interval of two to three hours.

If the red cells of the four group R sheep were lysed to the exclusion of those of the group O and i sheep, the serum was classified as anti-J. Conversely, if the red cells of the four group O sheep were lysed to the exclusion of those of the group R and i sheep, the serum was classified as anti-O. If the serum lysed the red cells of all group R and all group O sheep, but not those of group i, this was indicative of the presence of both anti-R and anti-O but, nevertheless, absorptions were performed in order to demonstrate the presence of each of two populations of antibody. A number of the cattle sera contained heterohemolysins for the red cells of all sheep and these heterohemolysins often obscured or masked hemolysis due to either or both anti-J and anti-O. Consequently, it was necessary to perform absorptions on sera of that order.

Where absorptions were necessary, each serum was diluted 1:4 and divided into three aliquots. One of the aliquots was absorbed twice with group R red cells using a ratio of red cells to serum of 1:2 for the first absorption and 1:1 for the second. The first absorption was run for 15 minutes at room temperature and the second for 30 minutes. It was then chilled at 4° C for 30 minutes just prior to centrifugation. The second aliquot was treated with group O red cells and the third with group i using the same procedure as that given for the aliquot treated with group R red cells. The three absorbed samples were heated for 30 minutes at 56° C and tested with the same panel of nine sheep bloods. The sera so treated were then classified on the basis of the antibodies remaining following the absorptions.

0.91 NaCl solution was used throughout these procedures.

RESULTS

As already mentioned, no further examples of natural, bovine antisheep O were encountered outside the immediate family of the Hereford bull whose serum served as the original source of anti-O used in the foregoing study (SPRAGUE 1958) as well as in the studies by RENDEL *et al.* (1954), SORENSEN *et al.* (1954) and RENDEL (1957). This particular bull, California Stanway 3rd registration number 4965229, was obtained from the University of California (U.C.) beef herd and was used in one of the experimental herds of this school.

Over the course of this study, 453 blood samples were collected from animals in the U.C. beef herds. Table 1 summarizes the results of the classification of these animals with respect to the occurrence of anti-J and anti-O, and also with respect to their **J-Oc** phenotypes, according to procedures given in the foregoing report (SPRAGUE 1958). Of the 453 samples, a total of 128 proved to have demonstrable anti-O and 17 of these 128 animals also possessed anti-J.

Table 2 is a summary of the inheritance data on anti-O. Because of the inhibitory effect of the phenotypes **J**, **JOc** and **Oc** on the appearance of anti-O, the critical matings (lines 4, 5 and 6 of Table 2) are restricted to parents of phenotype "—" in the J-Oc system. In these critical matings, it is seen that the inheritance of anti-O is consistent with the postulate of a single dominant gene. It remains to be seen whether more extensive data will bear out these observations.

The pattern of parentage information available for the entire herd (which included some offspring, one or both of whose parents was not tested) demonstrated that 100 animals with anti-O were related directly as descendants of either Dandy Domino the 46th 1573523 or California Stanway 2471801. The

TABLE 1

Classification of 453 Herefords (U.C. Beef) with respect to anti-J and anti-O antibodies and J and Oc soluble substances found in their sera

Phenotypic class	Number of animals	Number with anti-J and anti-O in serum	Number with anti-J in serum	Number with anti-O in serum	Number lacking both antibodies
JOc	29	0	0	0	29
J	93	0	0	0	93
Oc	58	0	33	0	25
—	273	17	60	111	85

TABLE 2

Summary of 277 matings with respect to the phenotype anti-O and the combined phenotypes **JOc, J, Oc** *here denoted as SSS (soluble specific substance) and the phenotype* "—" *(absence of SSS)*

Parental phenotypes		Offspring phenotypes		
		Anti-O	Non-anti-O	
SSS	SSS	—	—	SSS
1. non-anti-O ×	non-anti-O	7	3	42
SSS	—			
2. non-anti-O ×	non-anti-O	6	18	27
3. non-anti-O ×	anti-O	26	46	65
—	—			
4. anti-O ×	anti-O	12	6	0
5. anti-O ×	non-anti-O	9	17	0
6. non-anti-O ×	non-anti-O	0	11	2

— represents phenotype non-sss.

remaining 28 animals with anti-O were related to these same two lines of descent but were not directly related to the two sires.

Some anti-O animals have Oc-positive offspring as well as anti-O offspring. Assuming that the allele J^{Oc} (see preceding report) is a recessive, the segregation of Oc-positive animals from matings involving an anti-O parent indicates that the anti-O parent is heterozygous for the allele J^{Oc}. The question arises, is there a regular or necessary relationship between $J^{Oc}\ j$ heterozygotes and the phenotype anti-O? Since neither J-positive nor Oc-positive animals exhibit the phenotype anti-O, and there is no phenotypic test for $J^{Oc}\ j$ heterozygotes, an indirect test to determine a relationship between Oc and anti-O must be employed.

Let us call the class of J-negative-Oc-negative (i.e., $J^{Oc}\ j$) dams of Oc offspring X. Let us call the class of all J-negative-Oc-negative animals which are not X, Y (i.e., $J^{Oc}\ j$ and $j\ j$).

The proportion of X with anti-O and without anti-O may be compared with the proportion of Y with anti-O and without anti-O. If there is a necessary relationship between $J^{Oc}\ j$ heterozygotes and anti-O, the proportion of anti-O's among X should be different from the proportion of anti-O's among Y.

The data are X, anti-O 12; Y, anti-O 112; X, non-anti-O 9; Y, non-anti-O 148. The chi-square value for the significance of the difference between proportions is not regarded as significantly different and there is, as a consequence, no compelling evidence in favor of a regular relationship between the genotype $J^{Oc}\ j$ and the phenotype anti-O.

DISCUSSION

In the present study anti-J occurred in the serum of some J-negative cattle (**Oc** and "—" of Table 1) but not, of course, in the serum of J-positive cattle (**JOc** and **J**). On the other hand anti-O was only observed to occur within the phenotypic class "—". The reason for the "exclusion" of anti-O from J-positive phenotypes lies in the fact that all J-SSS sera tested to date exhibit weak cross reactions with anti-O. Erythrocytes from animals of these J-positive Oc-negative phenotypes will act in absorptions to remove anti-O antibodies. The converse situation, that is, cross reaction between Oc-SSS and anti-J, does not pertain since anti-J and Oc-SSS are frequently found to co-exist in the same serum. The cross reactions between J-SSS and anti-O are of such a degree that they rarely present a problem of classification in the routine inhibition tests.

The isoagglutinins of man are the most extensively studied natural antibodies because of their importance in human transfusions. The question as to whether or not these antibodies are inherited cannot be resolved as long as the very regular relationship between the agglutinins and the agglutinogens pertain. However, the cattle JOc system provides ideal conditions for the study of the genetic origin of isoantibodies because additional "degrees of freedom," as it were, are available. The critical classes of non-SSS with antibody and without antibody are commonly found as shown in Table 1. This critical observation permitted a study of the antibodies of segregants from matings of animals without detectable SSS with the results for anti-O shown in Table 2. The data avail-

able have also been examined with regard to anti-J and it has not been possible to formulate any generalization which might specifically account for the occurrence of this antibody fraction.

Two previous papers dealing directly with the genetic basis of normal antibody fractions are of particular interest.

The first, that of STUART *et al.* (1936), reported the investigation of normal rabbit sera containing agglutinins reactive with "group-specific A" substance of man. These workers investigated nine inbred strains of rabbits and concluded that "group-specific" agglutinins were inherited as the products of a mendelian recessive.

Further work by WHEELER *et al.* (1939), reveals that the presence of the *alpha* agglutinin may best be understood in terms of a human A (like) substance regularly found in *alpha* negative animals. Thus, this situation appears to be similar in form to that of the ABO system of man.

The second, that of STERN *et al.* (1956), presents statistical evidence for genetic control in mice of natural agglutinins for sheep erythrocytes.

At present it seems probable that although uncontrolled immunization will account for many examples of natural antibodies as reviewed by WEINER (1951) there may exist in addition cases of *bona fide* natural antibodies under direct genetic control.

SUMMARY

The origin of a natural antibody of cattle designated anti-O is ascribed to the action of a dominant gene.

The relationships between the phenotypes in the J-Oc system of cattle blood groups and anti-O are discussed.

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